Laboratory Safety

at the National Institutes of Health

NIH Division of Safety

Course Learning Objectives

This NIH Division of Safety training program includes a twenty-three (23) item written test that is given at the end of the presentation. The information presented on the test is covered during the program. In order to receive credit for attending this course you must complete the registration information and attempt the test. Participants successfully completing the test, registration information and evaluation form will be sent a certificate of attendance.

As a result of your participation in today's program, you should be able to do the following . . .

List the four primary routes of entry of potentially hazardous agents into the human body.

Distinguish between the different biological safety levels in terms of methods of transmission and work practice requirements.

Recognize key terms and certifications in the selection and use of personal protective equipment used in a biomedical research environment.

Define acronyms commonly used for information, container labeling, and exposure limits concerning chemical hazards in the workplace.

Recall the definitions of flash point and vapor pressure when assessing the hazards posed by flammable solvents.

State the expected response in the event of bloodborne pathogen exposure, biological or chemical spill, laboratory fire, or medical emergency at the NIH.

Describe and list steps that you can take to minimize the generation of potentially hazardous biological and chemical waste.

Identify the leading causes of laboratory fires and the NIH and the three elements in "The Fire Triangle."

Illustrate air flow patterns and features of biological safety cabinets and chemical fume hoods.

Special Note

We welcome non-NIH personnel at our programs on a space-available basis. The NIH does not certify or train non-NIH personnel for purposes of OSHA compliance. Training records for non-NIH personnel are not maintained.

Occupational Safety and Health Protection for Federal Employees

The Occupational Safety and Health Act of 1970, Executive Order 12196, and Title 29 Code of Federal Regulations part 1960 require the heads of federal agencies to establish programs to protect their employees from occupational safety and health hazards.

The designated safety and health official for the Department of Health and Human Services is
James F. Trickett
Deputy Assistant Secretary for Management and Acquisition
Room 4300, Cohen Building

Responsibilities of management are as follows:

1. To comply with safety and health standards issued under Section 6 of the Occupational Safety and Health Act of 1970, or develop alternate standards.
2. To develop procedures for responding to employee reports of unsafe and unhealthful working conditions.
3. To furnish approved personal protective and other safety equipment and enforce compliance with requirements for its use.
4. To inspect all workplaces at least annually with employee representatives and supervisors responsible for the workplaces.
5. To establish and enforce procedures to assure that employees are not subjected to restraint, interference, coercion, discrimination, or reprisal for exercising their rights under the provisions of the Occupational Safety and Health Program of the Department of Health and Human Services and its suborganizations.
6. To ensure that notices of serious unsafe or unhealthful working conditions found during inspections are posted near the locations where the conditions exist.
7. To establish procedures for the elimination of hazardous conditions, and to inform exposed employees of the plans for elimination the hazards. Imminent danger hazards will be corrected promptly.
8. To establish and maintain a management information system to record occupational accidents, injuries, illnesses, and their causes. The data contained in this system will be used to develop annual summaries of injuries, illnesses and useful preventive data. Summaries will be posted for a minimum of 30 days.
9. To establish and provide occupational safety and health training programs for employees as appropriate.
10. To establish occupational safety and health committees.

Responsibilities of employees are as follows:

1. To comply with occupational safety and health standards, rules, regulations and orders issued by the Department of Health and Human Services and its suborganizations which are applicable to their actions and conduct.
2. To use personal protective and other safety equipment provided for their protection.
3. To report all work-related accidents and illnesses to their supervisors.

Rights of employees and their representatives are as follows:

1. Employees shall be authorized and granted official time to participate in the Occupational Safety and Health Program.
2. Employees shall have access to safety manuals and records covering occupational safety and health standards and injury and illness statistics.
3. Employees shall be afforded the opportunity to comment on alternate occupational safety and health standards proposed by the Department of Health and Human Services and its suborganizations.
4. Employees shall have the right to report unsafe or unhealthful working conditions and to request to appropriate officials that safety and health inspections be conducted.
5. Additional information pertaining to the Occupational Safety and Health Program, its standards and procedures, the Federal Law, and other information on safety and health shall be available for review by employees through the NIH Division of Safety.

Occupational Safety and Health Branch	496-2346
Emergency Management Branch	496-1985
To report an emergency (on campus)	116
Using a TTY	496-0063
To report an emergency (off campus)	9-911
Environmental Protection Branch	496-3537
Radiation Safety Branch	496-5774
Occupational Medical Service	496-4411

To report unsafe acts or conditions notify:

- Your supervisor
- The NIH Division of Safety (496-2346)
- NIH Occupational Safety and Health Committee
  (Executive Secretary 496-2960)
- NIH BioSafety Committee
  (Executive Secretary 496-3353)
- NIH Radiation Safety Committee
  (Executive Secretary 496-5774)

Discrimination

Employees who exercise their rights under the provisions of the Occupational Safety and Health Program shall be protected from discrimination, restraint, interference, coercion or reprisal.

For additional information on Occupational Safety and Health at the NIH, please refer to Manual Issuance Chapter #1340—*Occupational Safety and Health Management.* (Available through your administrative office.)

NIH Division of Safety

This poster shall be displayed prominently where it can be easily read by all employees. The poster and any additions to it shall not be altered, defaced, or covered by any other materials

In The Event of a Fire

If you see smoke or flames. . .

Confine the fire by closing the door
Activate the nearest fire alarm
Report the fire

116	On Campus
9-911	Off Campus

State your name and exact location. Indicate where the problem is and if there are any injuries. Do not hang up until told to do so.

Evacuate the building by walking (not running) to the nearest FIRE EXIT and leave the building. *Do not use the elevators*. Once out of the building, do not re-enter until told to do so.

If you hear a fire alarm. . .

Shut off all open flames. Safely store hazardous materials. Leave your work location. Shut all doors behind you as you leave. Evacuate the building through the nearest FIRE EXIT. *Do not use elevators*.

Note to Supervisors:

Supervisors must ensure that all employees evacuate the building and that all individuals who may need help are assisted to safety.

In The Event of a Chemical Spill

Notify everyone in the room (or area) that a spill has occurred.
If needed, provide immediate first aid.

Eliminate potential sources of ignition, such as lights, motors, bunsen burners, etc.

Leave the room (or area) immediately and close the door behind you.

Notify the fire department:
116 On Campus
9-911 Off Campus
State your name and exact location
Indicate the chemical and amount spilled, if known
Report any injuries or special circumstances

First Aid for Chemical Contamination. . .
Go to the nearest eyewash station or safety shower
Flush the contaminated area with large volumes of water
While flushing, remove any clothing which may have been contaminated
Continue flushing until the fire department arrives

The fire department will continue first aid treatment upon arrival and will transport anyone who has been injured.

Do not permit anyone to re-enter the area until the appropriate authorities determine that the area is safe.

Chemical spills are a simple fact of life in a research laboratory. The NIH Fire Department has the requisite training, experience and equipment to handle laboratory spills; they are here to help you.

3 Emergency Steps To Take In the Event of a Potential HIV Exposure

1

Immediately initiate first aid at the work site.

Contaminated skin should be vigorously scrubbed for 10 minutes using a povidone iodine solution (such as Betadine) and copious amounts water.

Contaminated eyes and mucous membranes should be irrigated for 15 minutes using normal saline or water.

2

Notify your supervisor, if he or she is immediately available. Otherwise, go on to step three.

3

Report to the Occupational Medical Service (OMS) Building 10, Room 6C306. Phone 496-4411.

When OMS is closed, contact the Clinical Center Operator at 496-4567 to notify an OMS physician.

OCCUPATIONAL MEDICAL SERVICE(OMS), NIH Division of Safety

Information and Assistance

Emergency/Ambulance Response

On campus, except Building 10	**116**
Building 10 only	
Critical medical situations	**111**
Other emergencies	**116**
Off-campus buildings .	**9-911**

Program Locations

Workers' Compensation Office	496-9822
Building 10 Room 6C408	
Cardiopulmonary Resuscitation Training	
Building 31 Room B2B63	
Information	496-4111
Registration	496-7638
Employee Assistance Program	496-3164
Building 31 Room B2B57	

Health Unit Locations

Building 10 Main Health Unit

Room 6C306	496-4411
M-F 7:30a.m.-10:00p.m.	
After hours; HIV related incidents	496-1211
Clinical Center page operator	

Building 13 Clinic

Room G904		496-9278
M/W/TH/F	7:30a.m.-4:00p.m.	
Tuesday	7:30a.m.-2:30p.m.	

Westwood Clinic

Room 11		496-7638
M/W/F	8:00a.m.-4:30p.m.	
Tuesday	8:00a.m.-2:30p.m.	
Thursday	Closed	

We Can Provide

- Pre-employment medical evaluation
- Emergency medical evaluation to determine fitness for duty
- Evaluation and treatment for occupational injuries and illnesses
- Allergy testing and desensitization for work related allergies
- Evaluation and stabilization of personal medical emergencies
- Medical clearance to return to work if required by OMS or your supervisor
- Confidential counseling and referral through the Employee Counseling Service for emotional, alcohol, drug, and job related problems
- Psychiatric consultations
- Periodic medical surveillance for employees with occupational exposure to health hazards
- Health education programs including CPR training
- Health promotion programs including blood pressure checks, influenza vaccination, desensitization injections prescribed by your physician and provided by you for non-work related allergies
- Referral for personal health care to either community resources or appropriate NIH research studies when available
- Consultation on disability retirement applications

We Cannot

- Provide medical evaluation or treatment for non-emergent personal medical problems. We are only authorized to provide temporary relief for emergent non-occupational illness until you can see your own physician
- Interfere in any way with treatments being provided by your private physician
- Immunize members of your family
- Extend health service to Federal employees who are not working at NIH (except for emergencies)
- Provide any of the following services, except when ordered by an OMS physician:
  - ☐ Physical examinations
  - ☐ X-rays
  - ☐ Blood studies requested by your physician
  - ☐ Prescription drugs
  - ☐ Physiotherapy treatments ordered by your private physician
  - ☐ Dental care

OCCUPATIONAL MEDICAL SERVICE(OMS)
NIH Division of Safety

Care for Work-Related Injuries

Employees should report promptly to the Occupational Medical Service (OMS) for care and treatment of all injuries occurring while at work

If you are not able to report the injury in person because you are incapacitated at home or at a hospital, notify OMS and your supervisor by telephone as soon as possible

Should you require medical treatment from a private physician, the OMS will advise you and provide the necessary forms for your physician to submit a report

The OMS will also provide you with the official "Employees' Notice of Injury" form that is required to report an injury

Failure to submit reports promptly may jeopardize your receiving benefits to which you may be entitled

Questions related to Federal employees' compensation regulations and benefits may be directed to the OMS Workers Compensation Office (496-9822). Questions dealing with the provision of medical services may be directed to OMS staff at 496-4411

Thumbnail Sketches

Retrovirus Exposure Surveillance Program (RESP) provides both routine and post exposure testing for employees who may be exposed to HIV-1 through research involving HIV-infected blood, tissues and body fluids from infected animals, or in working with patients. Employees participating in the RESP program include research and clinical laboratory staff, and health care workers including nurses, phelbotomists, and physicians.

Hepatitis B Immunization Program focuses on protecting NIH employees against potential infection with the hepatitis B virus (HBV). The vaccine is offered before you begin work in an area which may include the potential exposure to hepatitis B virus (HBV). A blood test is offered before, during and annually after the hepatitis B vaccine is given. Employees enrolled in this program include health care workers such as doctors, nurses and phelbotomists; safety employees, and others working with the HBV or human blood and body fluids in the laboratory setting.

Animal Exposure Surveillance Program is designed for employees who work with any animal or in areas where animals are housed. The specifics of the program depend upon the type of potential exposure and the work area. Employees invited to participate include animal handlers, cage washers, and veterinarians.

Asbestos Surveillance Program is designed to provide regular health monitoring for employees who disturb asbestos in the course of their job. Employees who are encouraged to enroll in this program include maintenance engineers and insulators.

Respiratory Protection Program is a surveillance program for employees who are required to wear respirators to perform duties. Participants include asbestos workers, some animal handlers and fire fighters.

Notes

U.V. off when room occupied.
Leave running, or 5 min

Add 250 ml water
Bag autoclave in Bag in tray
50 minutes slow exhaust
Shelf for fume hood.

Summary of Recommended Biosafety Levels for Infectious Agents

Biosafety Level	Agents	Practices	Safety Equipment (Primary Barriers)	Facilities (Secondary Barriers)
1	Not known to cause disease in healthy adults.	Standard Microbiological Practices	None required	Open bench top sink required
2	Associated with human disease, hazard = auto-inoculation, ingestion, mucous membrane exposure	BSL-1 practice plus: • Limited access • Biohazard warning signs • "Sharps" precautions • Biosafety manual defining any needed waste decontamination or medical surveillance policies	Primary barriers = Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials; PPEs: laboratory coats; gloves; face protection as needed	BSL-1 plus: Autoclave available
3	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences	BSL-2 practice plus: • Controlled access • Decontamination of all waste • Decontamination of lab clothing before laundering • Baseline serum	Primary barriers = Class I or II BCSs or other physical containment devices used for all manipulations of agents; PPEs: protective lab clothing; gloves; respiratory protection as needed	BSL-2 plus: • Physical separation from access corridors • Self-closing, double-door access • Exhausted air not recirculated • Negative airflow into laboratory
4	Dangerous/exotic agents which pose high risk of life-threatening disease, aerosol-transmitted lab infections; or related agents with unknown risk of transmission	BSL-3 practices plus: • Clothing change before entering • Shower on exit • All material decontaminated on exit from facility	Primary barriers = All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air-supplied, positive pressure personnel suit	BSL-3 plus: • Separate building or isolated zone • Dedicated supply/exhaust, vacuum, and decon systems • Other requirements outlined in the text

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The term "containment" is used in describing safe methods for managing infectious agents in the laboratory environment where they are being handled or maintained. The purpose of containment is to reduce or eliminate exposure of laboratory workers, other persons, and the outside environment to potentially hazardous agents.

Primary containment, the protection of personnel and the immediate laboratory environment from exposure to infectious agents, is provided by both good microbiological technique and the use of appropriate safety equipment. The use of vaccines may provide an increased level of personal protection. Secondary containment, the protection of the environment external to the laboratory from exposure to infectious materials, is provided by a combination of facility design and operational practices. Therefore, the three elements of containment include laboratory practice and technique, safety equipment, and facility design. The risk assessment of the work to be done with a specific agent will determine the appropriate combination of these elements.

Laboratory Practice and Technique. The most important element of containment is strict adherence to standard microbiological practices and techniques. Persons working with infectious agents or potentially infected materials must be aware of potential hazards, and must be trained and proficient in the practices and techniques required for handling such material safely. The director or person in charge of the laboratory is responsible for providing or arranging for appropriate training of personnel.

Each laboratory should develop or adopt a biosafety or operations manual which identifies the hazards that will or may be encountered, and which specifies practices and procedures designed to minimize or eliminate risks. Personnel should be advised of special hazards and should be required to read and to follow the required practices and procedures. A scientist trained and knowledgeable in appropriate laboratory techniques, safety procedures, and hazards associated with handling infectious agents must direct laboratory activities.

When standard laboratory practices are not sufficient to control the hazard associated with a particular agent or laboratory procedure, additional measures may be needed. The laboratory director is responsible for selecting additional safety practices, which must be in keeping with the hazard associated with the agent or procedure.

Laboratory personnel, safety practices, and techniques must be supplemented by appropriate facility design and engineering features, safety equipment, and management practices.

Safety Equipment (Primary Barriers). Safety equipment includes biological safety cabinets (BSCs), enclosed containers, and other engineering controls designed to remove or minimize exposures to hazardous biological materials. The biological safety cabinet (BSC) is the principal device used to provide containment of infectious splashes or aerosols generated by many microbiological procedures. Three types of biological safety cabinets (Class I, II, III) used in microbiological laboratories are described and illustrated in Appendix A. Open-fronted Class I and Class II biological safety cabinets are primary barriers which offer significant levels of protection to laboratory personnel and to the environment when used with good microbiological techniques. The Class II biological safety cabinet also provides protection from external contamination of the materials (e.g., cell cultures, microbiological stocks) being manipulated inside the cabinet. The gas-tight Class III biological safety cabinet provides the highest attainable level of protection to personnel and the environment.

An example of another primary barrier is the safety centrifuge cup, an enclosed container designed to prevent aerosols from being released during centrifugation. To minimize this hazard, containment controls such as BSCs or centrifuge cups must be used for handling infectious agents that can be transmitted through the aerosol route of exposure.

Safety equipment also may include items for personal protection such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Personal protective equipment is often used in combination with biological safety cabinets and other devices which contain the agents, animals, or materials being worked with. In some situations in which it is impractical to work in biological safety cabinets, personal protective equipment may form the primary barrier between personnel and the infectious materials. Examples include certain animal studies, animal necropsy, agent production activities, and activities relating to maintenance, service, or support of the laboratory facility.

Facility Design (Secondary Barriers). The design of the facility is important in providing a barrier to protect persons working inside and outside of the laboratory within the facility, and to protect persons or animals in the community from infectious agents which may be accidentally released from the laboratory. Laboratory management is responsible for providing facilities commensurate with the laboratory's function and the recommended biosafety level for the agents being manipulated.

The recommended secondary barrier(s) will depend on the risk of transmission of specific agents. For example, the exposure risks for most laboratory work in Biosafety Level 1 and 2 facilities will be direct contact with the agents, or inadvertent contact exposures through contaminated work environments. Secondary barriers in these laboratories may include separation of the laboratory work area from public access, availability of a decontamination facility (e.g., autoclave), and handwashing facilities.

As the risk for aerosol transmission increases, higher levels of primary containment and multiple secondary barriers may become necessary to prevent infectious agents from escaping into the environment. Such design features could include specialized ventilation systems to assure directional air flow, air treatment systems to decontaminate or remove agents from exhaust air, controlled access zones, airlocks as laboratory entrances, or separate buildings or modules for isolation of the laboratory. Design engineers for laboratories may refer to specific ventilation recommendations as found in the *Applications Handbook for Heating, Ventilation, and Air-Conditioning (HVAC)* published by the American Society of Heating, Refrigerating, and Air-Conditioning Engineers (ASHRAE).[2]

Biosafety Levels. Four biosafety levels (BSLs) are described which consist of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities. Each combination is specifically appropriate for the operations performed, the documented or suspected routes of transmission of the infectious agents, and for the laboratory function or activity.

The recommended biosafety level(s) for the organisms in Section VII (Agent Summary Statements) represent those conditions under which the agent can ordinarily be safely handled. The laboratory director is specifically and primarily responsible for assessing risks and for appropriately applying the recommended biosafety levels. Generally, work with known agents should be conducted at the biosafety level recommended in Section VII. When specific information is available to suggest that virulence, pathogenicity, antibiotic resistance patterns, vaccine and treatment availability, or other factors are significantly altered, more (or less) stringent practices may be specified.

Biosafety Level 1 practices, safety equipment, and facilities are appropriate for undergraduate and secondary educational training and teaching laboratories, and for other facilities in which work is done with defined and characterized strains of viable microorganisms not known to cause disease in healthy adult humans. *Bacillus subtilis, Naegleria gruberi,* and infectious canine hepatitis virus are representative of those microorganisms meeting these criteria. Many agents not ordinarily associated with disease processes in humans are, however, opportunistic pathogens and may cause infection in the young, the aged, and immunodeficient or immunosuppressed individuals. Vaccine strains which have undergone multiple *in vivo* passages should not be considered avirulent simply because they are vaccine strains.

Biosafety Level 1 represents a basic level of containment that relies on standard microbiological practices with no special primary or secondary barriers recommended, other than a sink for handwashing.

Biosafety Level 2 practices, equipment, and facilities are applicable to clinical, diagnostic, teaching and other facilities in which work is done with the broad spectrum of indigenous moderate-risk agents present in the community and associated with human disease of varying severity. With good microbiological techniques, these agents can be used safely in activities conducted on the open bench, provided the potential for producing splashes or aerosols is low. Hepatitis B virus, the salmonellae, and *Toxoplasma* spp. are representative of microorganisms assigned to this containment level. Biosafety Level 2 is appropriate when work is done with any human-derived blood, body fluids, or tissues where the presence of an infectious agent may be unknown. (Laboratory personnel working with human-derived materials should refer to the *Bloodborne Pathogen Standard*[187] for specific, required precautions).

Primary hazards to personnel working with these agents relate to accidental percutaneous or mucous membrane exposures, or ingestion of infectious materials. Extreme precaution with contaminated needles or sharp instruments must be emphasized. Even though organisms routinely manipulated at BSL2 are not known to be transmissible by the aerosol route, procedures with aerosol or high splash potential that may increase the risk of such personnel exposure must be conducted in primary containment equipment, or devices such as a BSC or safety centrifuge cups. Other primary barriers should be used as appropriate, such as splash shields, face protection, gowns, and gloves.

Secondary barriers such as handwashing and waste decontamination facilities must be available to reduce potential environmental contamination.

Biosafety Level 3 practices, safety equipment, and facilities are applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents with a potential for respiratory transmission, and which may cause serious and potentially lethal infection. *Mycobacterium tuberculosis*, St. Louis encephalitis virus, and *Coxiella burnetii* are representative of microorganisms assigned to this level. Primary hazards to personnel working with these agents relate to autoinoculation, ingestion, and exposure to infectious aerosols.

At Biosafety Level 3, more emphasis is placed on primary and secondary barriers to protect personnel in contiguous areas, the community, and the environment from exposure to potentially infectious aerosols. For example, all laboratory manipulations should be performed in a BSC or other enclosed equipment, such as a gas-tight aerosol generation chamber. Secondary barriers for this level include controlled access to the laboratory and a specialized ventilation system that minimizes the release of infectious aerosols from the laboratory.

Biosafety Level 4 practices, safety equipment, and facilities are applicable for work with dangerous and exotic agents which pose a high individual risk of life-threatening disease, which may be transmitted via the aerosol route, and for which there is no available vaccine or therapy. Additionally, agents with a close or identical antigenic relationship to Biosafety Level 4 agents should also be handled at this level. When sufficient data are obtained, work with these agents may continue at this level or at a lower level. Viruses such as Marburg or Congo-Crimean hemorrhagic fever are manipulated at Biosafety Level 4.

The primary hazards to personnel working with Biosafety Level 4 agents are respiratory exposure to infectious aerosols, mucous membrane exposure to infectious droplets, and autoinoculation. All manipulations of potentially infectious diagnostic materials, isolates, and naturally or experimentally infected animals pose a high risk of exposure and infection to laboratory personnel, the community, and the environment.

The laboratory worker's complete isolation of aerosolized infectious materials is accomplished primarily by working in a Class III BSC or a full-body, air-supplied positive-pressure personnel suit. The Biosafety Level 4 facility itself is generally a separate building or completely isolated zone with complex, specialized ventilation and waste management systems to prevent release of viable agents to the environment.

The laboratory director is specifically and primarily responsible for the safe operation of the laboratory. His/her knowledge and judgment are critical in assessing risks and appropriately applying these recommendations. The recommended biosafety level represents those conditions under which the agent can ordinarily be safely handled. Special characteristics of the agents used, the training and experience of personnel, and the nature or function of the laboratory may further influence the director in applying these recommendations.

Animal Facilities. Four biosafety levels are also described for activities involving infectious disease work with experimental mammals. These four combinations of practices, safety equipment, and facilities are designated *Animal Biosafety Levels 1, 2, 3, and 4*, and provide increasing levels of protection to personnel and the environment.

Clinical Laboratories. Clinical laboratories, especially those in health care facilities, receive clinical specimens with requests for a variety of diagnostic and clinical support services. Typically, the infectious nature of clinical material is unknown, and specimens are often submitted with a broad request for microbiological examination for multiple agents (e.g., sputa submitted for "routine," acid-fast, and fungal cultures). It is the responsibility of the laboratory director to establish standard procedures in the laboratory which realistically address the issue of the infective hazard of clinical specimens.

Except in extraordinary circumstances (e.g., suspected hemorrhagic fever), the initial processing of clinical specimens and identification of isolates can be done safely at Biosafety Level 2, the recommended level for work with bloodborne pathogens such as hepatitis B virus and HIV. The containment elements described in Biosafety Level 2 are consistent with the *Occupational Exposure to Bloodborne Pathogens Standard*[187] from the Occupational Safety and Health Administration (OSHA), that requires the use of specific precautions with **all** clinical specimens of blood or other potentially infectious material (Universal Precautions).[43] Additionally, other recommendations specific for clinical laboratories may be obtained from the National Committee for Clinical Laboratory Standards.[134]

Biosafety Level 2 recommendations and OSHA requirements focus on the prevention of percutaneous and mucous membrane exposures to clinical material. Primary barriers such as biological safety cabinets (Class I or II) should be used when performing procedures that might cause splashing, spraying, or splattering of droplets. Biological safety cabinets should also be used for the initial processing of clinical specimens when the nature of the test requested or other information is suggestive that an agent readily transmissible by infectious aerosols is likely to be present (e.g., *M. tuberculosis*), or when the use of a biological safety cabinet (Class II) is indicated to protect the integrity of the specimen.

The segregation of clinical laboratory functions and limiting or restricting access to such areas is the responsibility of the laboratory director. It is also the director's responsibility to establish standard, written procedures that address the potential hazards and the required precautions to be implemented.

Importation and Interstate Shipment of Certain Biomedical Materials. The importation of etiologic agents and vectors of human diseases is subject to the requirements of the Public Health Service Foreign Quarantine regulations. Companion regulations of the Public Health Service and the Department of Transportation specify packaging, labeling, and shipping requirements for etiologic agents and diagnostic specimens shipped in interstate commerce (see Appendix D).

The U. S. Department of Agriculture regulates the importation and interstate shipment of animal pathogens and prohibits the importation, possession, or use of certain exotic animal disease agents which pose a serious disease threat to domestic livestock and poultry (see Appendix E).

Laboratory Biosafety Level Criteria

The essential elements of the four biosafety levels for activities involving infectious microorganisms and laboratory animals are summarized in Tables 1 and 2 (see pages 14 and 15). The levels are designated in ascending order, by degree of protection provided to personnel, the environment, and the community.

Biosafety Level 1

Biosafety Level 1 is suitable for work involving well-characterized agents not known to cause disease in healthy adult humans, and of minimal potential hazard to laboratory personnel and the environment. The laboratory is not necessarily separated from the general traffic patterns in the building. Work is generally conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not required nor generally used. Laboratory personnel have specific training in the procedures conducted in the laboratory and are supervised by a scientist with general training in microbiology or a related science.

The following standard and special practices, safety equipment and facilities apply to agents assigned to Biosafety Level 1:

A. *Standard Microbiological Practices*

1. Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments or work with cultures and specimens are in progress.

2. Persons wash their hands after they handle viable materials and animals, after removing gloves, and before leaving the laboratory.

3. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the work areas where there is reasonable likelihood of exposure to potentially infectious materials. Persons who wear contact lenses in laboratories should also wear goggles or a face shield. Food is stored outside the work area in cabinets or refrigerators designated and used for this purpose only.

4. Mouth pipetting is prohibited; mechanical pipetting devices are used.

5. All procedures are performed carefully to minimize the creation of splashes or aerosols.

6. Work surfaces are decontaminated at least once a day and after any spill of viable material.

7. All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method, such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are to be placed in a durable, leakproof container and closed for transport from the laboratory. Materials to be decontaminated at off-site from the laboratory are packaged in accordance with applicable local, state, and federal regulations, before removal from the facility.

8. An insect and rodent control program is in effect.

B. *Special Practices*: None

C. *Safety Equipment* (Primary Barriers)

1. Special containment devices or equipment such as a biological safety cabinet are generally not required for manipulations of agents assigned to Biosafety Level 1.

2. It is recommended that laboratory coats, gowns, or uniforms be worn to prevent contamination or soiling of street clothes.

3. Gloves should be worn if the skin on the hands is broken or if a rash exists.

4. Protective eyewear should be worn for anticipated splashes of microorganisms or other hazardous materials to the face.

D. *Laboratory Facilities* (Secondary Barriers)

1. Each laboratory contains a sink for handwashing.

2. The laboratory is designed so that it can be easily cleaned. Rugs in laboratories are not appropriate, and should not be used because proper decontamination following a spill extremely difficult to achieve.

3. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

4. Laboratory furniture is sturdy. Spaces between benches, cabinets, and equipment are accessible for cleaning.

5. If the laboratory has windows that open, they are fitted with fly screens.

Biosafety Level 2

Biosafety Level 2 is similar to Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs in that (1) laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists, (2) access to the laboratory is limited when work is being conducted, (3) extreme precautions are taken with contaminated sharp items, and (4) certain procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment.

The following standard and special practices, safety equipment, and facilities apply to agents assigned to Biosafety Level 2:

A. *Standard Microbiological Practices*

1. Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments are in progress.

2. Persons wash their hands after they handle viable materials and animals, after removing gloves, and before leaving the laboratory.

3. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the work areas. Persons who wear contact lenses in laboratories should also wear goggles or a face shield. Food is stored outside the work area in cabinets or refrigerators designated for this purpose only.

4. Mouth pipetting is prohibited; mechanical pipetting devices are used.

5. All procedures are performed carefully to minimize the creation of splashes or aerosols.

6. Work surfaces are decontaminated at least once a day and after any spill of viable material.

7. All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method, such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are to be placed in a durable, leakproof container and closed for transport from

the laboratory. Materials to be decontaminated at off-site from the laboratory are packaged in accordance with applicable local, state, and federal regulations, before removal from the facility.

8. An insect and rodent control program is in effect.

B. *Special Practices*

1. Access to the laboratory is limited or restricted by the laboratory director when work with infectious agents is in progress. In general, persons who are at increased risk of acquiring infection or for whom infection may be unusually hazardous are not allowed in the laboratory or animal rooms. For example, persons who are immunocompromised or immunosuppressed may be at risk of acquiring infections. The laboratory director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.

2. The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet specific entry requirements (e.g., immunization) enter the laboratory or animal rooms.

3. When the infectious agent(s) in use in the laboratory require special provisions for entry (e.g., immunization), a hazard warning sign incorporating the universal biohazard symbol is posted on the access door to the laboratory work area. The hazard warning sign identifies the infectious agent, lists the name and telephone number of the laboratory director or other responsible person(s), and indicates the special requirement(s) for entering the laboratory.

4. Laboratory personnel receive appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine or TB skin testing).

5. When appropriate, considering the agent(s) handled, baseline serum samples for laboratory and other at-risk personnel are collected and stored. Additional serum specimens may be collected periodically, depending on the agents handled or the function of the facility.

6. A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read and to follow instructions on practices and procedures.

7. Laboratory personnel receive appropriate training on the

potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates, or additional training as necessary for procedural or policy changes.

8. A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels. Needles and syringes or other sharp instruments should be restricted in the laboratory for use only when there is no alternative, such as parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles. Plasticware should be substituted for glassware whenever possible.

    a. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.

    b. Syringes which re-sheathe the needle, needle-less systems, and other safe devices should be used when appropriate.

    c. Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Containers of contaminated needles, sharp equipment, and broken glass are decontaminated before disposal, according to any local, state, or federal regulations.

9. Cultures, tissues, or specimens of body fluids are placed in a container that prevents leakage during collection, handling, processing, storage, transport, or shipping.

10. Laboratory equipment and work surfaces should be decontaminated with an appropriate disinfectant on a routine basis, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination by infectious materials. Contaminated equipment must be decontaminated according to any local, state, or federal

regulations before it is sent for repair or maintenance or packaged for transport in accordance with applicable local, state, or federal regulations, before removal from the facility.

11. Spills and accidents which result in overt exposures to infectious materials are immediately reported to the laboratory director. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

12. Animals not involved in the work being performed are not permitted in the lab.

C. *Safety Equipment* (Primary Barriers)

1. Properly maintained biological safety cabinets, preferably Class II, or other appropriate personal protective equipment or physical containment devices are used whenever:

    a. Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.

    b. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory if sealed rotor heads or centrifuge safety cups are used, and if these rotors or safety cups are opened only in a biological safety cabinet.

2. Face protection (goggles, mask, faceshield or other splatter guards) is used for anticipated splashes or sprays of infectious or other hazardous materials to the face, when the microorganisms must be manipulated outside the BSC.

3. Protective laboratory coats, gowns, smocks, or uniforms designated for lab use are worn while in the laboratory. This protective clothing is removed and left in the laboratory before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). All protective clothing is either disposed of in the laboratory or laundered by the institution; it should never be taken home by personnel.

4. Gloves are worn when handling infected animals and when hands may contact infectious materials, contaminated surfaces or equipment. Wearing two pairs of gloves may be appropriate; if a spill or splatter occurs, the hand will be protected after the contaminated glove is removed. Gloves are disposed of when contaminated, removed when work with infectious materials is completed, and are not worn outside the laboratory. Disposable gloves are not washed or reused.

D. *Laboratory Facilities* (Secondary Barriers)

1. Each laboratory contains a sink for handwashing.

2. The laboratory is designed so that it can be easily cleaned. Rugs in laboratories are not appropriate, and should not be used because proper decontamination following a spill is extremely difficult to achieve.

3. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

4. Laboratory furniture is sturdy, and spaces between benches, cabinets, and equipment are accessible for cleaning.

5. If the laboratory has windows that open, they are fitted with fly screens.

6. A method for decontamination of infectious or regulated laboratory wastes is available (e.g., autoclave, chemical disinfection, incinerator, or other approved decontamination system).

7. An eyewash facility is readily available.

Biosafety Level 3

Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents which may cause serious or potentially lethal disease as a result of exposure by the inhalation route. Laboratory personnel have specific training in handling pathogenic and potentially lethal agents, and are supervised by competent scientists who are experienced in working with these agents.

All procedures involving the manipulation of infectious materials are conducted within biological safety cabinets or other physical containment devices, or by personnel wearing appropriate personal protective clothing and equipment. The laboratory has special engineering and design features.

It is recognized, however, that many existing facilities may not have all the facility safeguards recommended for Biosafety Level 3 (e.g. access zone, sealed penetrations, and directional airflow, etc.). In these circumstances, acceptable safety may be achieved for routine or repetitive operations (e.g. diagnostic procedures involving the propagation of an agent for identification, typing, and susceptibility testing) in Biosafety Level 2 facilities. However, the recommended Standard Microbiological Practices, Special Practices, and Safety Equipment for Biosafety Level 3 must be rigorously followed. The decision to implement this modification of Biosafety Level 3 recommendations should be made only by the laboratory director.

The following standard and special safety practices, equipment and facilities apply to agents assigned to Biosafety Level 3:

A. *Standard Microbiological Practices*

1. Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments are in progress.

2. Persons wash their hands after handling infectious materials and animals, after removing gloves, and when they leave the laboratory.

3. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the laboratory. Persons who wear contact lenses in laboratories should also wear goggles or a face shield. Food is stored outside the work area in cabinets or refrigerators designated for this purpose only.

4. Mouth pipetting is prohibited; mechanical pipetting devices are used.

5. All procedures are performed carefully to minimize the creation of aerosols.

6. Work surfaces are decontaminated at least once a day and after any spill of viable material.

7. All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method, such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are to be placed in a durable, leakproof container and closed for transport from the laboratory. Materials to be decontaminated at off-site

from the laboratory are packaged in accordance with applicable local, state, and federal regulations, before removal from the facility.

8. An insect and rodent control program is in effect.

B. *Special Practices*

1. Laboratory doors are kept closed when experiments are in progress.

2. The laboratory director controls access to the laboratory and restricts access to persons whose presence is required for program or support purposes. For example, persons who are immunocompromised or immunosuppressed may be at risk of acquiring infections. Persons who are at increased risk of acquiring infection or for whom infection may be unusually hazardous are not allowed in the laboratory or animal rooms. The director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.

3. The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential biohazard, who meet any specific entry requirements (e.g., immunization), and who comply with all entry and exit procedures, enter the laboratory or animal rooms.

4. When infectious materials or infected animals are present in the laboratory or containment module, a hazard warning sign, incorporating the universal biohazard symbol, is posted on all laboratory and animal room access doors. The hazard warning sign identifies the agent, lists the name and telephone number of the laboratory director or other responsible person(s), and indicates any special requirements for entering the laboratory, such as the need for immunizations, respirators, or other personal protective measures.

5. Laboratory personnel receive the appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine or TB skin testing).

6. Baseline serum samples are collected and stored for all laboratory and other at-risk personnel. Additional serum specimens may be collected periodically, depending on the agents handled or the function of the laboratory.

7. A biosafety manual is prepared or adopted. Personnel are

advised of special hazards and are required to read and to follow instructions on practices and procedures.

8. Laboratory personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates, or additional training as necessary for procedural changes.

9. The laboratory director is responsible for insuring that, before working with organisms at Biosafety Level 3, all personnel demonstrate proficiency in standard microbiological practices and techniques, and in the practices and operations specific to the laboratory facility. This might include prior experience in handling human pathogens or cell cultures, or a specific training program provided by the laboratory director or other competent scientist proficient in safe microbiological practices and techniques.

10. A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels. Needles and syringes or other sharp instruments should be restricted in the laboratory for use only when there is no alternative, such as parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles. Plasticware should be substituted for glassware whenever possible.

    a. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.

    b. Syringes which re-sheathe the needle, needle-less systems, and other safe devices should be used when appropriate.

    c. Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Containers of contaminated needles, sharp equipment, and broken glass should be decontaminated before disposal, according to any local, state, or federal regulations.

11. All manipulations involving infectious materials are conducted in biological safety cabinets or other physical containment devices within the containment module. No work in open vessels is conducted on the open bench.

12. Laboratory equipment and work surfaces should be decontaminated with an appropriate disinfectant on a routine basis, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination with infectious materials. Contaminated equipment should also be decontaminated before it is sent for repair or maintenance or package for transport in accordance with applicable local, state, or federal regulations, before removal from the facility. Plastic-backed paper toweling used on non-perforated work surfaces within biological safety cabinets facilitates clean-up.

13. Cultures, tissues, or specimens of body fluids are placed in a container that prevents leakage during collection, handling, processing, storage, transport, or shipping.

14. All potentially contaminated waste materials (e.g., gloves, lab coats, etc.) from laboratories or animal rooms are decontaminated before disposal or reuse.

15. Spills of infectious materials are decontaminated, contained and cleaned up by appropriate professional staff, or others properly trained and equipped to work with concentrated infectious material.

16. Spills and accidents which result in overt or potential exposures to infectious materials are immediately reported to the laboratory director. Appropriate medical evaluation, surveillance, and treatment are provided and written records are maintained.

17. Animals and plants not related to the work being conducted are not permitted in the laboratory.

C. *Safety Equipment* (Primary Barriers)

1. Properly maintained biological safety cabinets are used (Class II or III - see Appendix A) for all manipulation of infectious materials.

2. Outside of a BSC, appropriate combinations of personal protective equipment are used (e.g., special protective clothing, masks, gloves, face protection, or respirators), in combination with physical containment devices (e.g., centrifuge safety cups, sealed centrifuge rotors, or containment caging for animals).

3. This equipment must be used for manipulations of cultures and of those clinical or environmental materials which may be a source of infectious aerosols; the aerosol challenge of experimental animals; harvesting of tissues or fluids from infected animals and embryonated eggs, and necropsy of infected animals.

4. Face protection (goggles and mask, or faceshield) is worn for manipulations of infectious materials outside of a biological safety cabinet.

5. Respiratory protection is worn when aerosols cannot be safely contained (i.e, outside of a biological safety cabinet), and in rooms containing infected animals.

6. Protective laboratory clothing such as solid-front or wrap-around gowns, scrub suits, or coveralls must be worn in, and not worn outside, the laboratory. Reusable laboratory clothing is to be decontaminated before being laundered.

7. Gloves must be worn when handling infected animals and when hands may contact infectious materials and contaminated surfaces or equipment. Disposable gloves should be discarded when contaminated, and never washed for reuse.

D. *Laboratory Facilities* (Secondary Barriers)

1. The laboratory is separated from areas which are open to unrestricted traffic flow within the building. Passage through two sets of self-closing doors is the basic requirement for entry into the laboratory from access corridors or other contiguous areas. A clothes change room (shower optional) may be included in the passage way.

2. Each laboratory contains a sink for handwashing. The sink is foot, elbow, or automatically operated and is located near the laboratory exit door.

3. The interior surfaces of walls, floors, and ceilings are water resistant so that they can be easily cleaned. Penetrations in these surfaces are sealed or capable of being

sealed to facilitate decontamination.

4. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

5. Laboratory furniture is sturdy, and spaces between benches, cabinets, and equipment are accessible for cleaning.

6. Windows in the laboratory are closed and sealed.

7. A method for decontaminating all laboratory wastes is available, preferably within the laboratory (i.e, autoclave, chemical disinfection, incineration, or other approved decontamination method).

8. A ducted exhaust air ventilation system is provided. This system creates directional airflow that draws air from "clean" areas into the laboratory toward "contaminated" areas. The exhaust air is not recirculated to any other area of the building, and is discharged to the outside with filtration and other treatment optional. The outside exhaust must be dispersed away from occupied areas and air intakes. Laboratory personnel must verify that the direction of the airflow (into the laboratory) is proper.

9. The High Efficiency Particulate Air (HEPA)-filtered exhaust air from Class II or Class III biological safety cabinets is discharged directly to the outside or through the building exhaust system. If the HEPA-filtered exhaust air from Class II or III biological safety cabinets is to be discharged to the outside through the building exhaust air system, it is connected to this system in a manner (e.g., thimble unit connection)[136] that avoids any interference with the air balance of the cabinets or building exhaust system. Exhaust air from Class II biological safety cabinets may be recirculated within the laboratory if the cabinet is tested and certified at least every twelve months.

10. Continuous flow centrifuges or other equipment that may produce aerosols are contained in devices that exhaust air through HEPA filters before discharge into the laboratory.

11. Vacuum lines are protected with liquid disinfectant traps and HEPA filters, or their equivalent, which are routinely maintained and replaced as needed.

12. An eyewash facility is readily available.

SAFET
NOTES

Minimizing Sharps Injuries in Biomedical Research Laboratories

Background

Since 1981, 1365 puncture wounds have been reported by NIH employees to the Occupational Medical Service (OMS)[1]. Approximately one out of every seven such injuries occurred in personnel not directly involved in patient care activities, such as researchers, technicians, animal handlers, or housekeepers. Of these non-patient care related puncture injuries, 20% were caused by sharp objects other than needles (e.g., broken Pasteur pipettes).

"Sharps" are defined as needles, scalpels, pipettes, broken plastic or glassware, and other instruments or devices capable of cutting or piercing the skin. Even sterile sharps are inherently hazardous because they can induce a traumatic percutaneous injury (e.g., cut to finger while putting a sterile scalpel blade on a handle). Non-sterile sharps pose an even greater occupational health risk to support service, research and health care personnel, because percutaneous injuries from contaminated sharps may introduce pathogens, hazardous chemicals or radioactive materials into the body.

There are over thirty thousand different chemicals found in the 2500 NIH research laboratory rooms; radioactive materials are used in 80% of these laboratories, and infectious agents are manipulated in nearly 50%.

Examples

Descriptions of sharps injuries documented through the NIH accident reporting system[1] illustrate the spectrum of this problem:

- "Animal care technician stuck while recapping a needle after anesthetizing a monkey."
- "Researcher punctured hand with needle while injecting a mouse."
- Housekeeper punctured hand with pipette contaminated with radioactive material while removing waste bag from laboratory."
- "Visiting scientist lacerated thumb while removing pipette from standard fluid culture bottle containing inactivated HIV."
- "Special volunteer worker cut finger with razor blade while handling an ultracentrifuge tube containing DNA."

Between 1981 and 1989, research scientists reported[1] 11% of NIH's sharps injuries, while technicians accounted for an additional 17%. Needles and syringes used in reconstituting antibiotics, shearing DNA, lysing cells, removing banded plasmid DNA from ultracentrifuge tubes, or as liquid transfer devices accounted for some injuries. Cuts with razor blades, broken pipettes, microtome blades and other sharps actually accounted for the vast majority (i.e., 80%) of the research-related percutaneous injuries.

Support services personnel sustained 14% of the reported sharps injuries. Most occurred while handling bags of general waste into which sharps had been inappropriately discarded (e.g., pipetes or needles *not* placed into an impervious sharps container).

The remaining 58% of the reported percutaneous exposures involved nursing personnel; of these, 20% were needle sticks that occurred while attempting to recap a needle, and 19% occurred while trying to stuff "just one more" into a sharps container.

Blood-borne Pathogens

Sharps injuries have a variety of outcomes, ranging from minor localized infections to generalized adverse health effects. Blood is the primary source of Hepatitis B virus (HBV) and human immunodeficiency virus (HIV), among other bloodborne pathogens, in the occupational setting[2]. Over 1200 people working in 250 NIH laboratories are currently registered with the Division of Safety as working with human blood and body fluids. All NIH personnel who handle human blood or body fluids are urged to consider the Hepatitis B vaccine program available through OMS.

For Assistance with	Contact	Telephone
Sharps disposal, Pathogen Registration, Procedural reviews, Laboratory surveys	**BID Safety Consultant** Safety Operations Section	**496-2346**
Hepatitis B Vaccine, Retrovirus Exposure Surveillance Program, Accident reporting	**Occupational Medical Service** (Bldg 10, 6C303; 8 am-11 pm, M-F)	**496-4411**
Sharps Containers (see NIH Supply Catalog, p. 85)	**Self Service Stores** Building 10	**496-2051**
	Building 31	**496-4430**
	Building 35	**496-4095**

There are approximately six million health care workers in the U.S. who handle blood, of which around 18,000 become infected annually with Hepatitis B virus[3]. When persons sustain sharps injuries involving HBV positive materials, up to 30% become infected. However, of workers who experience occupationally-related infections, fewer than 20% have previously reported a discrete percutaneous injury involving known infectious materials, suggesting a mucous membrane, skin or other exposure route.

Research on the human immunodeficiency virus has expanded rapidly at the NIH; more than 400 researchers currently work in 85 laboratories registered for work with human retroviruses. This has intensified concerns among biomedical research, health care and support service personnel regarding their potential risk of occupational exposure to HIV[4,5,6,7]. Of the 19 documented occupationally-related infections with HIV (including 2 laboratory personnel) in the biomedical research and health care settings in the U.S., **13 involved contaminated sharps**[3]. Between April 1988 and November 1989, eighty-eight exposures to materials potentially contaminated with HIV have been reported to OMS by NIH employees, of which thirty-three involved sharps.

Medical Prevention and Monitoring

All suspected injuries and exposures involving a retrovirus and/or human blood or body fluid should be reported to OMS immediately.

The preventive services and medical monitoring programs are available from the Occupational Medical Service include:

1) Immunization: Research and health care personnel who handle human blood or body fluids are encouraged to avail themselves of the Hepatitis B virus vaccine. Contact OMS for information.

2) Surveillance: Biomedical research and health care personnel working directly with human retroviruses or with human blood, tissue and body fluids should contact OMS regarding the Retrovirus Exposure Surveillance Program (RESP). RESP is the NIH medical surveillance program designed to monitor workers with potential occupational exposure to HIV.

Prudent Practices and Procedures

The following practices and procedures are strongly recommended for persons working with human blood and body fluids and most human pathogens[2,8]:

1) Use devices other than a needle and syringe to perform laboratory procedures. Mechanical pipetting devices, blunt canulas, and small-bore Tygon tubing may be used as substitutes for sharps in many situations. BID Safety and Health Consultants will offer alternative suggestions for the safe manipulation of materials. Call 496-2346 for assistance.

2) Minimize handling sharps. Do not recap, bend, break or otherwise manipulate needles by hand. Needles and syringes should be discarded intact. Use forceps or a Hemostat if a needle **must** be removed.

3) Discard all sharps in a puncture-resistant container, such as a Sharpsafe[9]; locate these as close to the work area as is practical. Do not overfill or force sharps into these containers, because it increases the possibility of sustaining a puncture wound. Seal the container when it is three-fourths full by snapping the lid closed. Place it into a Medical Pathological Waste (MPW) burn box.

4) Transport all blood samples and materials containing infectious agents in closed leak-proof containers. Contact your BID Safety Consultant at 496-2346 for assistance.

5) Follow **universal precautions**[2] when handling blood and body fluids, by wearing gloves and other protective clothing appropriate for the procedure/task being performed. Gloves will not prevent puncture wounds; therefore use extreme care when handling sharps. Wash hands thoroughly after each procedure/task, and immediately after any skin contamination with blood or body fluids, and after removal of gloves.

6) Report all sharps injuries to OMS.

7) Attend **Working Safely with HIV in the Research Laboratory** and other seminars offered by the Occupational Safety and Health Branch. Call 496-2346 for information.

[1-9] References are available from your BID Safety Consultant

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Effective Use of Biological Safety Cabinets (BSCs)

Biological safety cabinets (BSCs) are primary containment devices that are designed to provide for protection of the worker and the environment as well as provide a work environment free of extraneous contaminants. The effectiveness of the BSC is directly dependent on the manner in which users perform their work.

Understanding how the cabinet works:

- The effectiveness of the BSC is a function of three separate directional airflows: inward from the room through the front grille (providing personal protection with this air barrier); downward through a high efficiency particulate air (HEPA) filter onto the work surface (providing product protection); and out of the cabinet through an exhaust HEPA filter (providing environmental protection).

- Disrupting these airflow patterns reduces cabinet effectiveness. Such disruptions may be caused by: rapidly moving your arms in and out of the BSC; people walking rapidly behind you across the face of the cabinet; down-drafts from room ventilation systems; cross drafts from open laboratory windows or doors; blockage of the front or rear grilles; or placement of bulky materials or equipment within the cabinet.

- A biological safety cabinet is not a chemical fume hood! You will be at increased risk of exposure to potentially hazardous chemical vapors if you use volatile chemicals in a biological safety cabinet (rather than a fume hood), because air is recirculated within the cabinet and back into the laboratory.

- Boxes stored on top of BSCs may block the filters; excess materials inside the cabinet will disrupt the air flow and may compromise the containment capability of the BSC. Good laboratory housekeeping reduces the chance of contamination.

General suggestions:

- Wear gloves to protect your hands from the microbes with which you work; gloves also protect tissue cultures from microbial contamination by your hands. Wash your hands thoroughly after working in your BSC.

- A buttoned lab coat or back-closing gown will protect your street clothes; remove lab coats before leaving your laboratory.

- Please contact the Occupational Safety and Health Branch when you wish to purchase a new BSC. Also contact them if you wish to move your BSC, so that arrangements can be made for cabinet decontamination and/or recertification.

- Obtain approval from the Radiation Safety Branch before using volatile radioactive materials in your BSC (496-5774).

Operational suggestions:

1. Turn on the BSC five minutes before beginning work to remove any air-borne contaminants. Wipe the cabinet work surface with 70% ethanol. Also wipe off each item you place into the BSC to minimize the introduction of extraneous microorganisms.

a. Keep the rear exhaust and the front air intake grilles clear from research objects or notebooks; arms should not rest across the front grille.

b. Cover the work surface with plastic-backed absorbent lab toweling to capture splatter and facilitate spill clean-up, being careful not to block the grilles. Replace toweling when overtly contaminated or at the end of the work to help assure a clean work environment.

c. Arrange materials in the BSC to segregate contaminated and clean items. Avoid movement of contaminated items over clean ones. Remember, "work from clean to dirty."

d. Perform work at least four inches behind the front air intake grille.

e. Wipe down cabinet work surfaces when work is completed.

2. Follow standard good microbiological practices and procedures as described in the CDC/NIH publication, Biosafety in Microbiological and Biomedical Laboratories.

a. Use mechanical pipetting aids. Never mouth pipette.

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b. Use horizontal pipette discard pans containing an effective disinfectant solution inside the BSC. Do not use vertical pipette discard canisters placed on the floor outside the cabinet.

c. Discard bags should be placed in the rear corner of the cabinet, not taped to the front of the cabinet.

d. If you hold open tubes and bottles as horizontal as possible inside the cabinet, it is not necessary to flame them. An open flame inside the BSC will create turbulence in the airflow and may compromise the clean environment. Open flames have been known to ignite fires in cabinets. Prolonged heat build-up may also damage the integrity of the filters.

4. Move your arms slowly to minimize disruption of the fragile air barrier when you remove items or introduce new items into the BSC.

5. If you use a piece of equipment that creates air turbulence in the BSC (e.g., centrifuge, vortex mixer), place the equipment in the back third of the cabinet work surface; stop other work while the equipment is operating.

6. Protect the building vacuum system from biohazards by placing a cartridge filter (contact your Safety and Health Consultant for ordering information) between the collection vessel and the vacuum shut-off valve in the cabinet. Disposable suction flasks usually have suitable built-in filters. Place disinfectant into the collection flask to decontaminate the fluids. Chemically-decontaminated fluids may be poured down laboratory drains.

7. Clean up all spills in the cabinet immediately. Wipe down the working surface with 70% ethanol; wait 3-5 minutes before resuming work. If a spill occurs which runs through the grille, decontaminate the tray beneath the work surface with sufficient chemical disinfectant. This can be emptied through the tray drain into a bucket or other collection vessel. Call the Safety Operations Section, OSHB, for assistance (496-2346.)

8. Remove gloves and dispose in biohazard bag or MPW box. Remove lab coat and wash hands thoroughly before leaving the lab.

Waste management:

1. Place all biohazardous wastes into autoclavable bags before removal from the BSC.

2. If the materials are to be decontaminated in a steam autoclave, add 500 ml of water before sealing the bag. Otherwise, the bag must be opened to allow adequate contact with steam during autoclaving.

3. Autoclaved or chemically-decontaminated materials may be placed into a Medical Pathological Waste (MPW) box or discarded as general waste.

4. Biohazardous waste material that has not been decontaminated may be discarded in an MPW box. Do not attempt to autoclave MPW or other cardboard boxes.

Radioactive spill clean-up:

The following points need to be considered in the event of a radioactive spill in your BSC:

1. Spill cleanup is the responsibility of the investigator.

a. Wear protective gloves and a lab coat.

b. Radioactive cleaners (particularly the newer foams) are useful for spill clean-up inside a BSC.

2. If the spill involves a biologically or chemically hazardous material in addition to the radioactive material, the Safety Operations Sections, OSHB, should be contacted for guidance on handling the non-radioactive components of the spill.

3. The front grille of a biological safety cabinet can be removed for decontamination of the grille itself or of the tray underneath. Contact the Safety Support Section, OSHB, for advice before attempting to remove any part of your cabinet.

a. The contaminated tray area can be wiped clean if it is easily accessible.

b. Otherwise, the tray can be flushed with an appropriate detergent solution which can be emptied through the tray drain into a properly labelled radioactive waste carboy.

4. All waste generated in the radioactive spill cleanup process should be considered radioactive and should be appropriately labelled and disposed of via the NIH radioactive waste contractor.

For Assistance	**Contact**	**Telephone**
Use of the BSC	BID Safety Consultants	**496-2346**
Bio/Chem Safety Issues	Safety Operations Section	**496-2346**
Purchasing, Servicing or Moving a BSC	Safety Support Section	**496-3457**
Radiological Issues	Health Physicists	**496-5774**
Radioactive waste pick-up	Rad waste Contractor	**496-8100**

APPENDIX A

Biological Safety Cabinets

Biological Safety Cabinets (BSCs) are among the most effective, as well as the most commonly used primary containment devices in laboratories working with infectious agents. The three general types available (Class I, II, III) have performance characteristics and applications which are described in this appendix.

Properly maintained Class I and II BSCs, when used in conjunction with good microbiological techniques, provide an effective containment system for safe manipulation of moderate and high-risk microorganisms (Biosafety Level 2 and 3 agents). Both Class I and II BSCs have inward face velocities (75-100 linear feet per minute) that provide comparable levels of containment for laboratory workers and the immediate environment from infectious aerosols generated within the cabinet. Class II BSCs have the additional advantage of providing protection to the research material by high-efficiency particulate air (HEPA)-filtration of the air flow down across the work surface (vertical laminar flow). Class III cabinets offer the maximum protection to laboratory personnel, the community, and the environment because all hazardous materials are contained in a totally enclosed, ventilated cabinet.

CLASS I

(Note: Class I BSCs are no longer being manufactured on a regular basis; many have been replaced by Class II BSCs.)

The *Class I Biological Safety Cabinet* (Fig. 1) is a negative-pressure, ventilated cabinet usually operated with an open front and a minimum face velocity at the work opening of at least 75 linear feet per minute (lfpm). All of the air from the cabinet is exhausted through a HEPA filter either into the laboratory, or to the outside. The Class I BSC is designed for general microbiological research with low and moderate risk agents, and is useful for containment of mixers, blenders, and other equipment. These cabinets are *not* appropriate for handling research materials that are vulnerable to airborne contamination, since the inward flow of unfiltered air from the laboratory can carry microbial contaminants into the cabinet.

The Class I BSC can also be used with an installed front closure panel without gloves that will increase the inward flow velocity to approximately 150 lfpm. If such equipped cabinets are ducted to the outside exhaust, they may be used for toxic or radiolabelled materials used as an adjunct to microbiological research. Additionally, arm-length rubber gloves may be attached to the front panel with an inlet air pressure release for further protection.

CLASS II

The *Class II Biological Safety Cabinet* (Fig. 2) is designed with inward air flow at a velocity to protect personnel (75-100 lfpm), HEPA-filtered vertical laminar airflow for product protection, and HEPA-filtered exhaust air for environmental protection. Design, construction and performance standards for Class II BSCs, as well as a list of products that meet these standards, have been developed by and are available from the National Sanitation Foundation International,[136] Ann Arbor, Michigan. Utilization of this standard and list should be the first step in selection and procurement of a Class II BSC.

Class II BSCs are classified into two types (A and B) based on construction, air flow velocities and patterns, and exhaust systems. Basically, Type A cabinets are suitable for work with microbiological research *in the absence of* volatile or toxic chemicals and radionuclides, since air is recirculated within the work area. Type A cabinets may be exhausted through HEPA filters into the laboratory, or to the outside via a "thimble" connection to the exhaust ductwork.

Type B cabinets are further sub-typed into types B1, B2, and B3. A comparison of the design features and applications are found in Table 1 of this appendix. Type B cabinets are hard-ducted to the exhaust system, and contain negative pressure plena. These features, plus an increased face velocity of 100 lfpm, allow work to be done with toxic chemicals or radionuclides.

It is imperative that Class I and II biological safety cabinets are tested and certified *in situ* at the time of installation within the laboratory, at any time the BSC is moved, and at least annually thereafter. Certification at locations other than the final site may attest to the performance capability of the individual cabinet or model but does not supersede the critical certification prior to use in the laboratory.

As with any other piece of laboratory equipment, personnel must be trained in the proper use of the biological safety cabinets. Of particular note are those activities which may disrupt the inward directional airflow through the work opening of Class I and II cabinets. Repeated insertion and withdrawal of the workers' arms in and from the work chamber, opening and closing doors to the laboratory or isolation cubicle, improper placement or operation of materials or equipment within the work chamber, or brisk walking past the BSC while it is in use are demonstrated causes of the escape of aerosolized particles from within the cabinet. Class I and II cabinets should be located away from traffic patterns and doors. Fans, heating and air conditioning registers, and other air handling devices can also disrupt airflow patterns if located adjacent to the BSC. Strict adherence to recommended practices for the use of BSCs and proper placement in the laboratory are important in attaining the maximum containment capability of the equipment as is the mechanical performance of the equipment itself.

CLASS III

The *Class III Biological Safety Cabinet* (Fig. 3) is a totally enclosed, ventilated cabinet of gas-tight construction and offers the highest degree of personnel and environmental protection from infectious aerosols, as well as protection of research materials from microbiological contaminants. Class III cabinets are most suitable for work with hazardous agents that require Biosafety Level 3 or 4 containment.

All operations in the work area of the cabinet are performed through attached rubber gloves. The Class III cabinet is operated under negative pressure. Supply air is HEPA-filtered, and the cabinet exhaust air is filtered by two HEPA filters in series, or HEPA filtration followed by incineration, before discharge outside of the facility.

All equipment required by the laboratory activity, such as incubators, refrigerators, and centrifuges, must be an integral part of the cabinet system, The Class III cabinet must be connected to double-doored autoclaves and chemical dunk tanks to sterilize or disinfect all materials exiting the cabinet, and to allow supplies to enter the cabinet. Several Class III cabinets are therefore typically set up as an interconnected system.

POSITIVE-PRESSURE PERSONNEL SUIT

Personnel protection equivalent to that provided by Class III cabinets can also be obtained with the use of a one-piece, ventilated suit for the laboratory worker when working with Biosafety Level 3 or 4 agents in a "suit area" and using Class I or II BSCs. The personnel suit is maintained under positive pressure with a life support system to prevent leakage into the suit. In this containment system, the worker is isolated from the work materials.

The personnel suit area must be essentially equivalent to a large Class III cabinet. The area is entered through an air-lock fitted with airtight doors. A chemical shower is provided as a "dunk tank" to decontaminate the surfaces of the suit as the worker leaves the area. The exhaust air from the suit area is filtered by two HEPA filter units installed in series. The entire area must be under negative pressure.

As in the case with Class III BSCs, the gloves of the personnel suit are the most vulnerable component of the system, as they are subject to punctures by sharps or animal bites.

(Caution: Horizontal laminar flow "clean benches" are used in clinical, pharmaceutical, and laboratory facilities strictly for *product* protection. Since the worker sits in the immediate downstream exhaust from the "clean bench", this equipment must *never* be used for handling toxic, infectious, radioactive, or sensitizing materials.)

Figure 1. *Class I Biological Safety Cabinet.* A. front opening, B. work surface, C. window, D. exhaust plenum, E. HEPA filter.

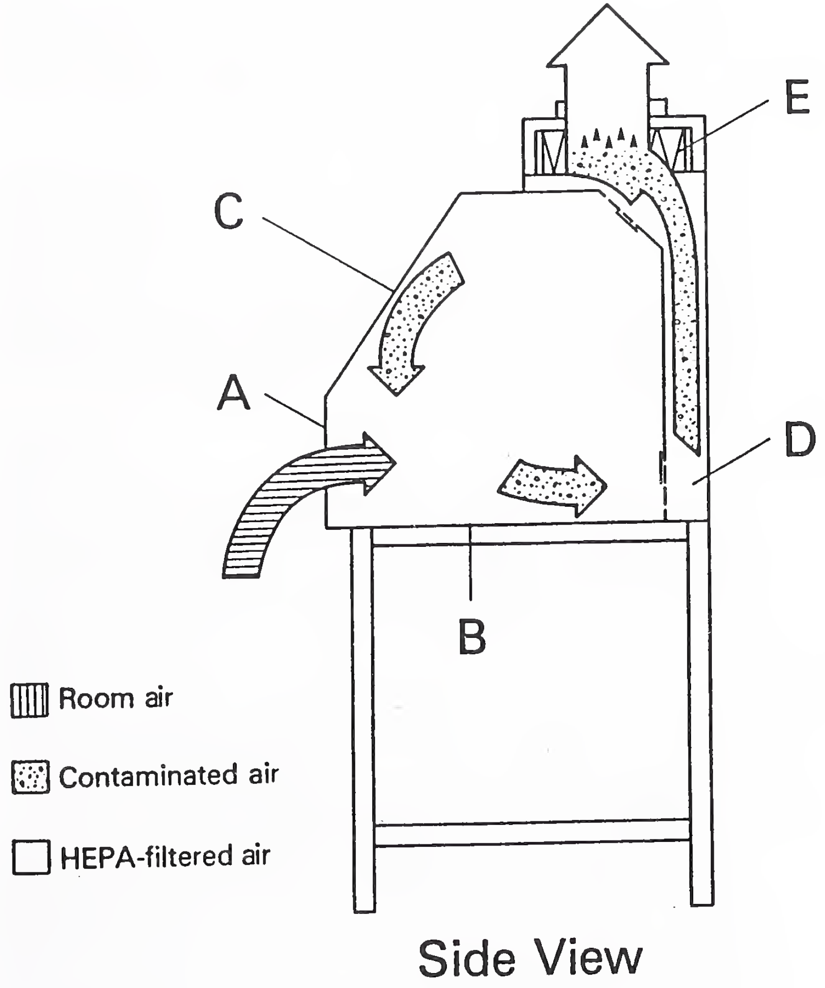
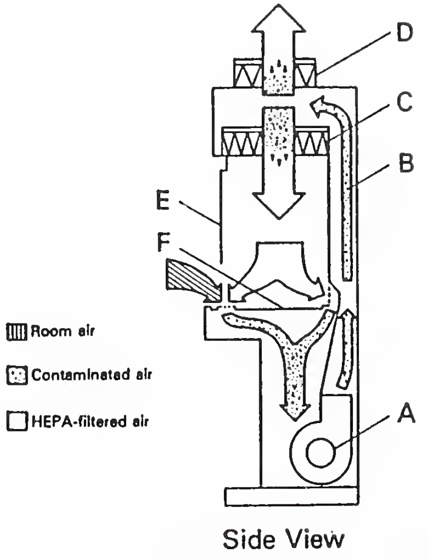


Figure 2(a). *Class II, Type A BSC.* A. blower, B. rear plenum, C. supply HEPA filter, D. exhaust, E. sash, F. work surface.

Figure 2(b). *Class II, Type B1 BSC.* A. blowers, B. supply HEPA filters, C. sliding sash, D. positive pressure plenums, E. additional supply HEPA filter or back-pressure plate, F. exhaust HEPA filter, G. negative pressure exhaust plenum, H. work surface.



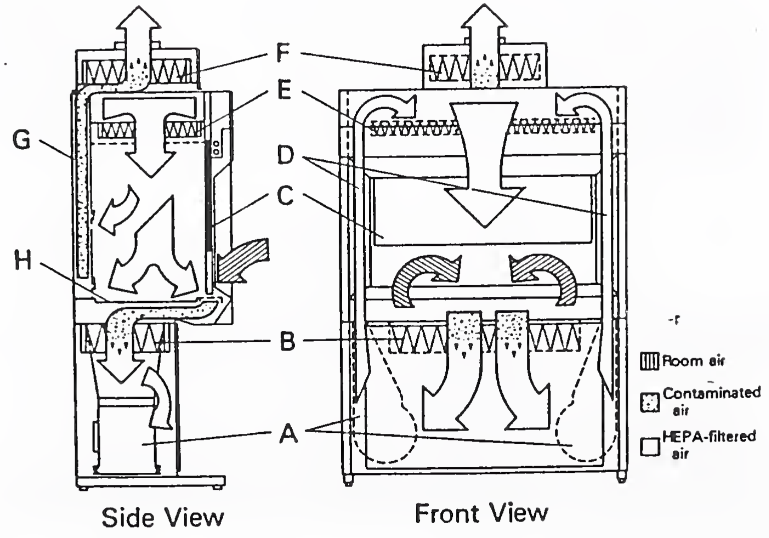
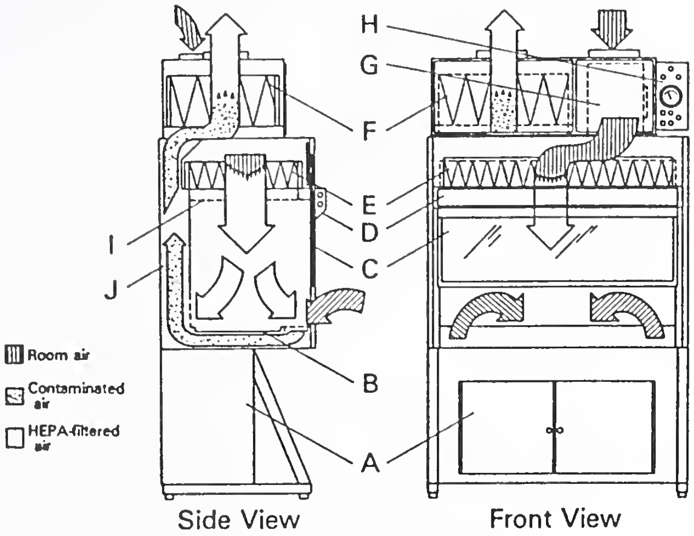


Figure 2(c). *Class II, Type B2 BSC.* A. storage cabinet, B. work surface, C. sliding sash, D. lights, E. supply HEPA filter, F. exhaust HEPA filter, G. supply blower, H. control panel, I. filter screen, J. negative pressure plenum.

Figure 2(d). *Table-top model of a Class II, Type B3 BSC.* A. front opening, B. sliding sash, C. light, D. supply HEPA filter, E. positive pressure plenum, F. exhaust HEPA filter, G. control panel, H. negative pressure plenum, I work surface.



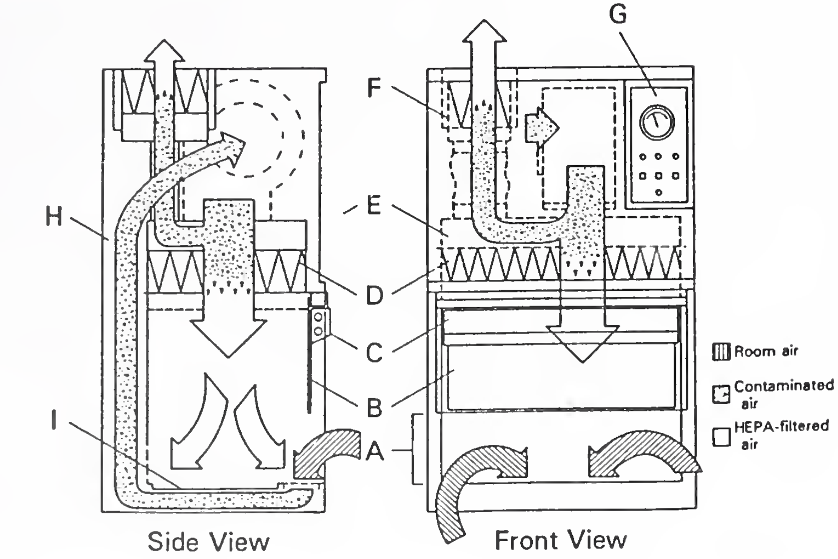
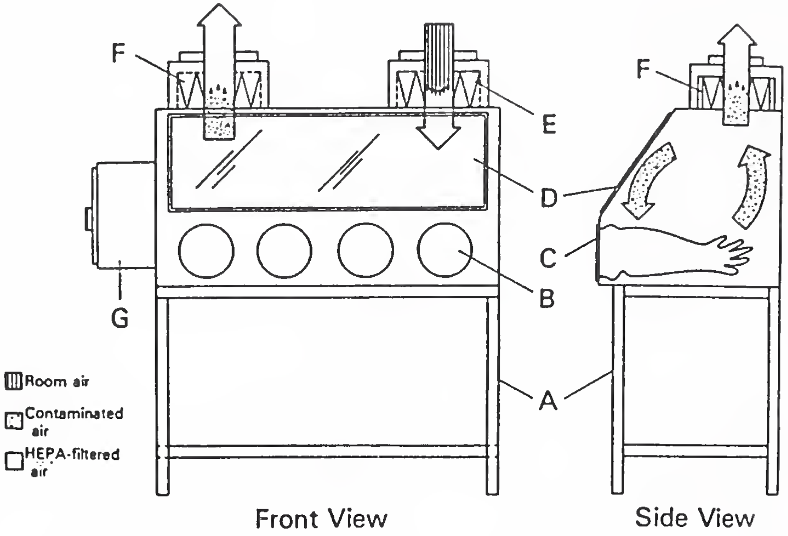
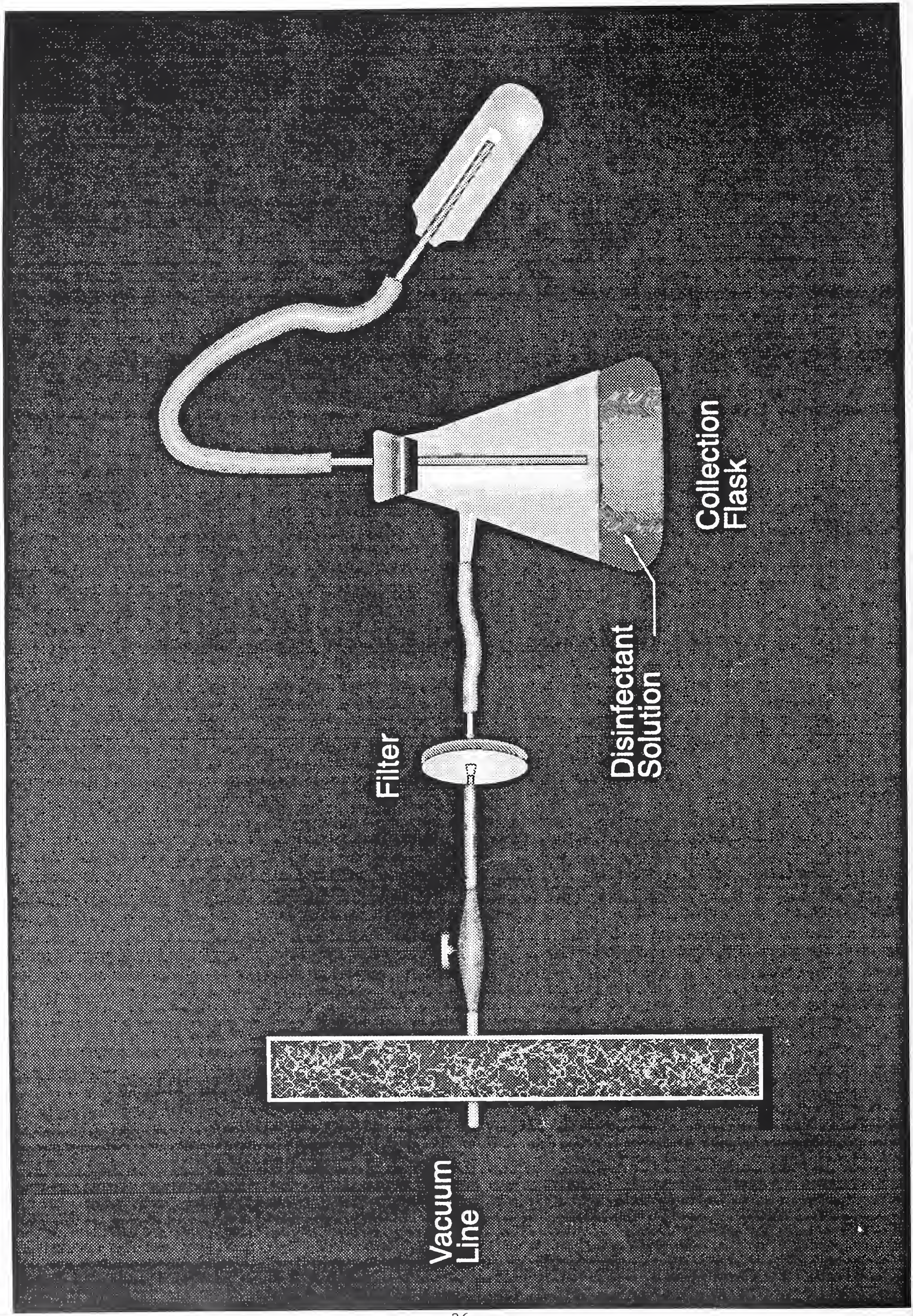


Figure 3. *Class III BSC.* A. stand, B. glove ports, C. O-ring for attaching arm-length gloves to cabinet, D. slopped glass viewing window, E. supply HEPA filter, F. exhaust HEPA filter (Note that the second exhaust HEPA filter required for Class III cabinets is not depicted in this diagram), G. double-ended autoclave.

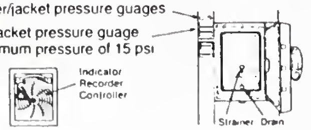



Vacuum Line
Filter
Disinfectant Solution
Collection Flask

Steam Autoclave Usage For Safety and Quality Control

Occupational Safety and Health Branch, Division of Safety

Routine Tasks in Care of Sterilizers



chamber/jacket pressure guages

check jacket pressure guage for minimum pressure of 15 psi

check temperature for 250°F (121°C) every load

Inspect drain strainer daily
Clean when blocked

Use Sterilization Indicators

- Mechanical recorder/indicators record the time-temperature profile attained during a cycle
- Visual indicators chemically measure one or more physical conditions of the autoclave cycle.
- Biological indicators (spore strips) are the only specific indicators of sterility
- Use indicators to determine the best treatment time

Notify Maintenance Engineering if These Troubles Persist:

- No steam.
- Valves leaking
- Door gasket deterioration
- Jacket pressure below 15 psi
- Temperature not reaching 250°F (121°C)
- Erratic temperature or pressure during cycle
- Steam escapes from around door during cycle.
- Water on floor or in chamber after a cycle
- Articles very wet after a Fast Exhaust and Dry cycle
- Excessive steam in area during cycle or after opening door

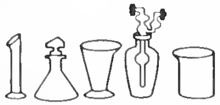
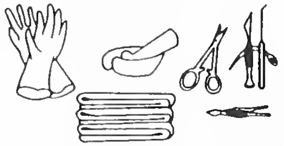
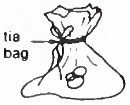
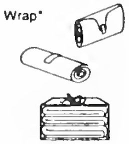
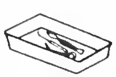
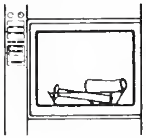
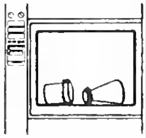
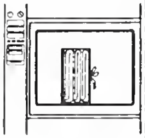
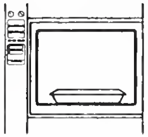
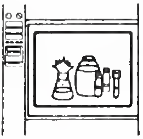
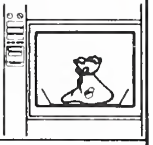
Maintenance Engineering
Phone Number ____________

Choose Proper Operating Conditions

Sterilization will only result when condition of time, temperature, pressure and humidity have been met.

Incorrect selection of time or exhaust cycle may damage the autoclave, cause liquid boil-over or break bottles.

Proper use of autoclave will minimize the chance of serious injury.

Typical Articles	Glassware 		Dry Items 		Liquids 	Biohazard Bags add 250 ml water 
Preparation	Dirty Place in metal pans on side or inverted	Clean Wash Rinse Wrap*	Fabrics Wrap* 	Instruments Clean & Dry Lay in Pan Wrap* 	Loosen caps or use vented closurs Fill no more than 75% capacity Use type 1 borosilicate glass	Place in metal pan Affix indicator if needed
Placement In Autoclave	Detergent & Water in Pan 	On side or inverted 	Separated, on edge 	Flat 	Upright 	In Pan 
Treatment Time (Minutes)	30	30	30-60	30-60	20-test tubes 40-1000ml Erl/flasks	50
Exhaust Cycle	Slow Exhaust	Fast Exhaust & Dry	Fast Exhaust & Dry	Fast Exhaust & Dry	Slow Exhaust (Liquids)	Slow Exhaust
Notes	Glassware with cracks or deep scratches may break		*Check reference for proper packaging methods		Hot bottles may explode Cool before moving	Avoid puncturing bags Dispose of waste properly

Cautions

1. Always wear goggles or faceshield, lab coat or apron, and heat-protective non-esbestos gloves when opening door or removing items from autoclave.
2. Open door slowly Beware of rush of steam
3. Open door only after chamber pressure returns to zero. Leave door open for several minutes to allow pressure to equalize and for materials to cool.
4. Do not mix loads which require different exposure times and exhaust
5. Materials that will melt (plastic lab wear) and block chamber exhaust drain should be placed in a shallow stainless steel autoclave pan

Need Help?

General Information:
Occupational Safety and Health Branch
496-2346

Waste Disposal Problems:
Environmental Protection Branch
496-7990

Reference:
"Using the Gravity Displacement Steam Autoclave in the Biomedical Laboratory"
DHHS/PHS/NIH/DS

Decontaminating Medical Pathological Waste (MPW) Contair in Biohazard Autoclave Bags

Investigators should add ~250 ml water to bag before closing and transporting to autoclave area.

Biohazard autoclave bags must indicate adequate decontamination before disposal as general waste.

- Use autoclave high strength polymer bags imprinted with process indicator.
- Attach autoclave tape if indicator is not present.
- Place bag in pan before autoclaving
- Repeat cycle if process indicator has not changed color to show evidence of steam processing.
- See "Waste Disposal at NIH" calendar for additional information.

MPW BURN BOX PROCEDURES

Packing

1. Double bag ALL waste
2. Close & seal each bag SEPARATELY
   with draw-tie closures
3. WRITE bldg. & lab room number on box label

Box Closing

1. Fold Flap A down into carton
2. Fold B Flaps over Flap A
3. Push Flap C down to lock with Flap A

NEW MPW BURN BOXES

The medical pathological waste (MPW) Burn Box has a new look. With the cooperative efforts of research personnel and support staff, the NIH Division of Safety, ORS, developed a safer and easier to use new MPW box. The new bright white box with blaze orange lettering replaces the old brown MPW box.

Putting the new box together is simpler because the bottom folds automatically into place and the specially designed 'T-Top' flaps interlock. Unlike the old design, the new MPW box does not require tape. Instructions are printed on each box.

The new MPW box comes as part of a kit that contains five boxes, ten bags and 12 plastic draw-tie closures. The new packaging system for MPW boxes makes them easier to purchase, requires less storage room and will also make it easier to comply with the new requirement to double bag all MPW waste. The MPW box kits are available through the NIH Self-Service Store in Building 35 and NIH Stock Catalog # 8115-00-L04-06800.

To prevent accidental incineration, do not use MPW boxes for moving or storing research records or equipment.

Your cooperation in the proper packaging of MPW Burn Boxes will make the disposal process safer and easier for everyone.

Agent: Herpesvirus simiae (B-virus)

B-virus is a naturally occurring alphaherpesvirus infecting free-living or captive *Macaca mulatta, M. fasicularis*, and other members of the genus. It is associated with acute vesicular oral lesions, as well as latent and often recrudescent infection.[193] Human infection has been documented in 25 instances, usually with a lethal outcome or serious sequelae from encephalitis.[7,92,145]

Although B-virus presents a potential hazard to laboratory personnel working with the agent, laboratory-associated human infections with B-virus have, with rare exceptions, been limited to those having direct contact with macaques. Primary macaque cell cultures, including commercially-prepared rhesus monkey kidney cells, occasionally are inapparently infected with B-virus and have been implicated in one human case.[92]

Sixteen fatal cases of human infections with B-virus have been reported.[193]

Laboratory Hazards: The highest risk of acquiring B-virus from macaques is through the bite of an infected monkey with active lesions. Contamination of broken skin or mucous membranes with oral, ocular, or genital secretions from animals with lesions, or experiencing clinically silent virus shedding, is also dangerous. Stability of viral infectivity on cages and other surfaces is not known, but the potential hazard must be recognized. The importance of aerosol exposure is thought to be minimal. Attenuated or avirulent strains have not been identified.

The agent also may be present in thoracic and abdominal viscera and nervous tissues of naturally infected macaques. These tissues, and the cultures prepared from them, are potential hazards.[196]

Recommended Precautions: Biosafety Level 2 practices and facilities are recommended for all activities involving the use or manipulation of tissues, body fluids, and primary tissue culture materials from macaques. Additional practices and personnel precautions, such as those detailed for Biosafety Level 3, are recommended for activities involving the use or manipulation of any material known to contain *Herpesvirus simiae*. *In vitro* propagation of the virus for diagnosis may be conducted under the same guidelines, but it would be prudent to confine manipulations of positive cultures which would contain high-titered virus to a Class 3 biosafety cabinet or BSL-4 facility, depending on the judgement of the laboratory director.

Biosafety Level 4 practices and facilities are recommended for activities involving the propagation and manipulation of production quantities or concentrates of *H. simiae*.

The wearing of gloves, masks, and laboratory coats is recommended for all personnel working with non-human primates -- especially macaques and other Old World species -- and for all persons entering animal rooms where non-human primates are housed. Any macaque colony not known to be free of B-virus infection should be presumed to be naturally infected. Guidelines are available for safely working with macaques and should be consulted.[39,150] Animals with oral lesions suggestive of active B-virus infection should be isolated and handled with extreme caution. Studies with animals experimentally infected with *H. simiae* should be conducted at ABSL-3.

Vaccines are not available for use in humans. Human to human transmission has only occurred in one case, suggesting that precautions should be taken with vesicle fluids, oral secretions, and conjunctival secretions of infected persons.[92]

Antiviral drugs have shown promise in the therapy of rabbits infected with *H. simiae*, and anecdotal observations suggest this may extend to man.[7,92] Because of the seriousness of infection with this virus, experienced medical personnel should be available for consultation to manage incidents involving exposure to the agent or suspected infections.

NIH MANUAL 3044-2
DATE: 2/9/93
REPLACES: none
ISSUING OFFICE: OIR/OD 496-4920

PROTECTION OF NIH PERSONNEL WHO WORK WITH NONHUMAN PRIMATES

A. PURPOSE

Established under this chapter is the NIH policy for protection of NIH personnel who work with or around nonhuman primates. The policy describes the requirements for training, supervision, compliance, personal protective equipment, medical surveillance, accident reporting, and wound care. The intent of this policy is to minimize the overall number of injuries, specifically bites and scratches, sustained by NIH employees, special volunteers, and visitors who work with nonhuman primates or enter nonhuman primate rooms. Contractors engaging in activities with nonhuman primates must have a program in place with policies and procedures equivalent to the program described herein. Project Officers for such contracts shall ensure that all contracts contain the equivalent components.

B. POLICY AND PROCEDURES

Training. Each individual regularly entering a nonhuman primate room shall have received introductory training prior to entry. Transient visitors will be supervised by someone with appropriate training. Those individuals having hands-on interaction with nonhuman primates must also have additional training in approaching and handling nonhuman primates. Certification by the first line supervisor that a basic level of performance has been achieved is required. The Training Coordinator, Office of Animal Care and Use (OACU/OD), is responsible for the development and management of the program for training intramural personnel to work safely and humanely with nonhuman primates.

Supervision and Compliance. All NIH employees, including special volunteers, guest workers, visitors, and contract personnel shall comply with procedures set forth in this policy statement. Immediate supervisors are responsible for ensuring that their employees follow established policy. Corrective action shall be taken by the immediate supervisor for failure to comply with the provisions of this policy.

PROTECTION OF NIH PERSONNEL WHO WORK WITH NONHUMAN PRIMATES

Continued failure to comply with requirements set forth in this policy shall be reported to the Scientific Director and/or Institute Director and may result in suspension of the privilege to use nonhuman primates in research protocols or other disciplinary action. The Institutional Official for Animal Welfare Assurance (i.e., Deputy Director for Intramural Research) shall be informed of such infractions and disciplinary actions taken.

Personal Protective Equipment. All individuals entering a nonhuman primate room must wear appropriate personal protective clothing and equipment which meets or exceeds the guidance established by the NIH Animal Research Advisory Committee (ARAC) (Appendix 1). Guidance on the selection and use of personal protective equipment for selected protocols will be provided upon request, by the Occupational Safety and Health Branch, Division of Safety (OSHB, DS). The NIH Institutional Biosafety Committee (NIH IBC) shall advise on appropriate protective measures, as needed, at the request of the Division of Safety. Individuals participating in protocols involving infectious disease agents must meet or exceed practices and procedures recommended in the CDC/NIH publication entitled *Biosafety in Microbiological and Biomedical Laboratories* and any additional stipulations placed on the protocol by the NIH IBC. Copies of THE CDC/NIH publication may be obtained by calling 496-2346. All support personnel, who have not been appropriately trained, (e.g. building engineers, pest controllers, safety and health personnel, etc.) entering nonhuman primate rooms must be accompanied by a responsible facility staff member who is knowledgeable in the behavior and handling of nonhuman primates.

Medical Surveillance. All persons having direct contact with nonhuman primates in the course of conducting research and all persons providing care must participate in the NIH Animal Exposure Surveillance Program (AESP). Transient visitors, who are required to enter a room housing nonhuman primates but do not have direct contact with the animals, are not required to participate in the AESP but are to wear single-use dust/mist masks in addition to other required protective clothing. All contractor employees having direct contact with nonhuman primates must participate in an AESP that is equivalent to that of the NIH and which shall be provided by their employer.

Accident Reporting. All accidents and injuries involving animals, animal wastes, or potentially contaminated equipment must be reported promptly to the first-line supervisor. An individual who sustains any injury must report to the Occupational Medical Service (OMS) as soon as possible. The Division of Safety will regularly review accident/injury reports and make accident information available to the Training Coordinator, OACU, who will ensure that training will address identified problem areas.

Wound Care. Eeach nonhuman primate facility manager or ICD veterinarian, as applicable, is responsible for maintaining an adequately stocked wound care kit. The kit includes materials for culturing a wound, inflicted by a nonhuman primate, for *Herpesvirus simiae* also known as Monkey B virus. The wound care kit must be located in an easily accessible area, and instructions for culturing wounds, first aid, and reporting to OMS must be prominently displayed. The facility manager or veterinarian, as applicable, is responsible for informing all research and animal care staff of the location of the wound care kit. The procedures to be followed in the event of a nonhuman primate bite or scratch are described in the NIH Animal Exposure Surveillance Program (AESP).

C. ADDITIONAL INFORMATION

For further information on this manual chapter, contact the NIH Office of Animal Care and Use (496-5424).

D. ADDITIONAL COPIES

Copies of this manual chapter may be obtained by completing Form NIH 414-5 and submitting it to the P&RB, DSS, ORS, in Bldg 31, Room B4BN08.

NIH MANUAL 3044-2 APPENDIX 1
DATE: 2/9/93
REPLACES: none
ISSUING OFFICE: OIR/OD 496-4920

PROTECTION OF NIH PERSONNEL WHO WORK WITH NONHUMAN PRIMATES

Minimum Requirements for Protective Clothing in Nonhuman Primate Rooms

Although research using animals entails some real and unavoidable risks, the goal of protective programs is to enable animal based research to go on in a safe and productive environment. Our goal is to provide a set of principles that can be applied to a variety of animal facilities. Two principles are at the base for all protective measures: accident avoidance based on understanding the physical risks entailed, as exemplified by avoiding bites, and scratches; and good personal hygiene based on knowledge of when contamination is most likely to occur, methods of avoidance, and decontamination, as exemplified by proper use of barrier methods and good hand washing practices.

For a protective program to be effective, workers must believe that the recommendations that are in place increase their safety significantly. The most important preventive strategy is education. Vigilance in animal handling, specific training of scientists and handlers in techniques for safe interaction with animals, proper use of protective clothing and equipment, and hand washing should be stressed. Any preventive measures that are put in place must be accompanied by an educational process that instructs all animal workers in the methods and rationale for each preventive measure. Protocols for emergency diagnosis and treatment of injury, especially possible B virus infection should be worked out ahead of time and all personnel instructed in their use. Workers who are knowledgeable about the risks and the protection offered by each device, article of clothing, and procedure should enthusiastically cooperate with the protection program.

All animal handlers, scientists, technicians and caretakers must be enrolled in the Animal Exposure Surveillance Program. This program classifies employees according to the species of animals to which they are exposed and provides appropriate surveillance and prophylaxis to these employees. Employees immunosuppressed by virtue of disease or treatment should be advised of their increased risks and should limit their exposure to animals.

NIH MANUAL 3044-2 APPENDIX 1
DATE: 2/9/93
REPLACES: none
ISSUING OFFICE: OIR/OD 496-4920

PROTECTION OF NIH PERSONNEL WHO WORK WITH NONHUMAN PRIMATES

Minimum Requirements for Protective Clothing in Nonhuman Primate Rooms

These requirements are not meant to cover all of the specific instances that might be encountered, or to limit the requirements of any facility, but to provide a framework of minimum standards for implementing a program tailored to the local conditions. Specific recommendations for a safe environment must be formulated to account for local conditions, and these may vary from one laboratory to the next even within a single facility. Exceptions to any of these requirements may be requested of the ICD ACUC. However, in these instances individuals must demonstrate adequate training and proficiency in appropriate techniques and the Division of Safety must concur with the exceptions. The NIH Institutional Biosafety Committee (IBC) will serve as the final review body, as needed. Specific minimum requirements for handling nonhuman primates have been extracted from the ACUC guidance document and are presented in Appendix 2.

NIH MANUAL 3044-2 APPENDIX 2
DATE: 2/9/93
REPLACES: None
ISSUING OFFICE: OIR/OD 496-4920

PROTECTION OF NIH PERSONNEL WHO WORK WITH NONHUMAN PRIMATES

Protective Clothing Requirements for Personnel in Nonhuman Primate Facilities

ACTIVITY	REQUIREMENTS
View animals in the primate room. No contact with the animals or the cages	Mucous membrane protection as appropriate*, street clothes covering, handwashing should be performed upon leaving the area and after any of the following activities.
Contact with a restraint device holding an awake animal	Mucous membrane protection as appropriate*, street clothes covering and light gloves
Transfer alert monkey using a stand-off method such as pole/collar technique or transfer cage	Mucous membrane protection as appropriate*, street clothes covering and light gloves.
Handling (e.g. hand catching or restraining) of an alert monkey.	Mucous membrane protection appropriate for high risk situations, street clothes covering and arm length bite protection gloves.
Physical contact with an anesthetized monkey.	Mucous membrane protection as appropriate*, street clothes covering and light gloves.
Cleaning cages.	Mucous membrane protection appropriate for high risk situations, dedicated clothing, dedicated shoes, and light gloves; dust-mist masks, if approved by OSHB, DS.
Physical contact with restrained alert monkey	Mucous membrane protection as appropriate*, street clothes covering and light gloves.
Physical contact with infant monkeys	Mucous membrane protection as appropriate*, street clothes covering and light gloves. As infants mature, heavier gloves may be required.
Perform portions of experiments in a laboratory that do not involve physical contact with monkey or body fluids.	Protection appropriate for non-animal aspects of research.
Handling awake New World Species	Mucous membrane protection as appropriate*, street clothes covering, protective gloves appropriate for the species and size of animal being handled. Larger species such as Cebus should be handled with the arm length bite protection gloves, while very small species such as marmosets may be handled with lighter weight protection.

* Mucous membrane protection should be appropriate to the potential for splash hazard. The level of mucous membrane protection should be detailed in facility standard operating procedures (SOP's). SOP's are subject to review and approval by the ICD ACUC with the concurrence of the Division of Safety with consult by the IBC as necessary. The degree of risk involved in entering an animal holding room or working with awake animals varies with the design of the facility, the species involved and the nature of the task being performed. Examples of high risk situations include any procedures which may aerosolize NHP wastes or body fluids, e.g., hosing down animal rooms, dental work or tracheal intubation, especially of macaques. Lower risks are associated with fully restrained animals, anesthetized animals (However, Ketamine increases salivation which is a major source of potential B virus contamination from macaques.), and increased distance from animals. In very low risk situations, e.g., entering hallways through which animals in restraint devices may briefly pass, mucous membrane protection may not be required. The future definition and availability of SPF animals may alter these requirements.

A QUICK GUIDE
WOUND CARE INSTRUCTIONS FOR EMPLOYEE'S HANDLING NON-HUMAN PRIMATES

Monkey Bites or Scratches

Report the injury to OMS - Building 10, Room 6C306, 496-4411. If closed go immediately to Suburban Hospital or initiate first aid yourself.

First Aid At The Worksite For Employee

SUPPLIES NEEDED:

2 Culture tubes per wound
2 Dacron Swabs per wound
Disposable gloves

1. With your gloved hand culture the fresh wound by twirling the cotton tip into the wound with gentle pressure.
2. Place cotton tip into culture tube - break off and secure, label, "First culture".
3. For skin injuries, cleanse wound with povidone iodine (betadine) & sterile sponge portion of surgical scrub. Do a ten (10) minute scrub.
4. For eye splashes irrigate eye with 0.9% NACL for a full fifteen (15) minutes.
5. Re-culture wound as before and label "Second culture".
6. Refrigerate cultures.
7. Report to OMS with cultures during next scheduled clinic hours.

Obtaining Cultures From Monkey

SUPPLIES NEEDED:

3 Culture tubes
3 Dacron Swabs
Phlebotomy Equipment
Disposable Gloves

1. Culture - right eye - left eye - mouth and label.
2. Obtain 4ml of blood from monkey and label "Serum from Monkey" with date & monkey #.
3. Make sure OMS has the name, phone #, building and room # of veterinarian in charge.

Notes

NATIONAL INSTITUTES OF HEALTH
DIVISION OF SAFETY
OCCUPATIONAL SAFETY AND HEALTH BRANCH
NIH Chemical Hygiene Plan

Introduction

In the laboratory, the use of hazardous chemicals is a necessary part of modern biomedical research science. In an effort to ensure the protection of laboratory personnel from the hazards associated with the use of hazardous chemicals, the Occupational Safety and Health Administration (OSHA) has promulgated a standard entitled *Occupational Exposures to Hazardous Chemicals in Laboratories* (29 CFR 1910.1450). Traditionally, OSHA health standards have been established to help protect industrial and manufacturing workers who may be exposed to significant quantities of (usually) a few hazardous chemicals over a working life time. In laboratories, the use of hazardous chemicals is generally limited to small quantities used on a short-term basis and in operations where the chemicals and procedures change frequently. In knowing this, OSHA has recognized the need for a standard which focuses on the unique nature of laboratory work.

The laboratory standard requires the development and implementation of a formal, written, and employee-accessible program referred to as a *Chemical Hygiene Plan*. As defined by OSHA, this plan must be "capable of protecting employees from health hazards associated with hazardous chemicals used in the laboratory".

The laboratory standard complements the provisions of the OSHA *Federal Hazard Communication Standard* (29 CFR 1910.1200). To comply with portions of the Hazard Communication standard, the NIH has a written program entitled the *NIH Hazard Communication Program* (NIH-HCP). The NIH-HCP provides for the identification and inventory of hazardous chemicals; the availability to employees of Material Safety Data Sheets for these chemicals; chemical container labeling; and employee training in hazard communication.

The *NIH Chemical Hygiene Plan* is consistent with the *NIH Hazard Communication Program*.

Additionally, the laboratory standard supersedes the provisions of all other OSHA health standards except for the permissible exposure limits found within the OSHA *Air Contaminates Standard* (29 CFR 1910.1000) and the substance-specific standards found in 29 CFR 1910, Subpart Z.

NIH Chemical Hygiene Plan Written Program

I Purpose

This plan outlines the information and services provided by the Occupational Safety and Health Branch, Division of Safety on the safe use, storage and disposal of the hazardous chemicals in the laboratory.

This written program is prepared in compliance with 29 CFR 1910.1450 "*Occupational Exposure to Hazardous Chemicals in Laboratories*".

II Scope

This plan applies to all laboratories and laboratory personnel of the NIH that use, store or handle hazardous chemicals.

Selected Definitions

Chemical Hygiene Plan: a written plan which establishes procedures and policies to protect laboratory personnel and other support staff from the potential adverse health effects associated with exposure to hazardous chemicals.

Chemical Hygiene Officer: a qualified individual who provides technical guidance in developing and implementing a chemical hygiene plan. At the NIH, the Chief of the Occupational Safety and Health Branch/ Division of Safety serves in this capacity.

Designated Area: a pre-determined and well labeled area in which carcinogens, reproductive toxins (teratogens/ embryotoxins), or other chemicals with significant acute or chronic toxicity are used/kept in the laboratory.

Laboratory Personnel: Any person working in an NIH laboratory who handles or uses potentially hazardous chemicals. At the NIH, visiting scientists, guest researchers, special volunteers, students and other similar personnel are included in the scope of the Chemical Hygiene Plan.

Hazardous Chemical: a substance which is recognized to have a measurable potential for adverse (acute or chronic) health effects in humans. The Federal Hazard Communication Standard provides additional guidance in determining the extent of the hazard presented by a chemical. For additional information, please refer to the NIH Hazard Communication Program.

ICD: Institutes Centers and Divisions. An abbreviation used at the NIH that refers to organizational and management structure.

Laboratory\Laboratory Scale\ Laboratory Use: a workplace in which relatively small quantities of hazardous chemicals (usually several) are used in a non-production basis, and in which the tasks performed are generally managed by one person.

Particularly Hazardous Substances: these include "select carcinogens", reproductive toxins and substances which have a high degree of acute toxicity.

Reproductive Toxins: Chemicals which affect an individuals reproductive ability including chromosomal damage (mutations) and/or has an adverse effect on a fetus (teratogenesis).

"Select Carcinogen": a substance regulated by OSHA as such, as designated by the National Toxicity Program (NTP) or the International Agency for Research on Cancer (IARC) as having a moderate to high potential for causing cancer in animal models. This distinction separates moderate to high risk carcinogens from those of slight to minimal risk when viewed in the context of their use in a laboratory.

III Responsibilities

The NIH Manual Issuance #1340: NIH Occupational Safety and Health Management outlines the scope, objectives, and responsibilities of NIH staff in implementing the NIH Occupational Safety and Health Management Program.

Additional information on the safe use of hazardous chemicals is provided in the NIH Hazard Communication Program.

An OSHB Safety and Health Specialist is assigned to each ICD to assist laboratory workers in matters relating to chemical safety.

IV Information and Training

Information on chemical safety in the laboratory is made available through participation in the OSHB course, Laboratory Safety at the National Institutes of Health. This three hour program provides an introduction to biomedical laboratory safety including information on the recognition, evaluation and control of biological, chemical, and physical hazards. Contact the OSHB at 496-2346 for a list of upcoming program dates.

The laboratory supervisor is responsible for providing health and safety information to his or her staff on the specific hazards found in a particular laboratory.

V Safe Work Practices with Hazardous Chemicals

Each laboratory supervisor should ensure that all laboratory personnel under their direction possess the requisite knowledge, training, and education to safely handle hazardous chemicals in the laboratory. All laboratory personnel are responsible for following the appropriate work practices when using hazardous chemicals.

Safe Work Practices With Hazardous Chemicals in the Laboratory

- It is prudent to minimize all chemical exposures and avoid an underestimation of the risk.
- Food, coffee, tea, cosmetics, medication belong *outside* the lab.
- Protect your clothes and exposed skin through the use of laboratory coats, gowns. Open-toed shoes, sandals, shorts and other apparel that leave exposed skin are not appropriate when handling potentially hazardous chemicals. Lab coats must not be worn outside of the laboratory.
- When handling hazardous chemicals, wear the appropriate gloves and eye/face protection *at all times*.
- Ensure *unrestricted* access to a suitable eyewash station and shower.
- After working with laboratory chemicals, carefully remove your gloves and thoroughly wash your hands and forearms.
- Use a chemical fume hood when having to open, pour, or handle chemicals that would otherwise enter the atmosphere without containment.
- Keep all doors to the laboratory closed when using the fume hood to ensure proper hood operation. Open laboratory doors can adversely affect hood performance.
- *Absolutely never* mouth pipet.
- Safely transport laboratory chemicals through the use of bottle carriers and suitable carts.
- In the event of a significant hazardous chemical spill *follow the hazardous material spill procedure immediately*.

Hazardous Material Spill Procedure

1. Leave room, close doors, and do not open windows.
2. Call the Fire Department: 116 On-campus/ 9-911 Off-campus
3. Wash any parts of the body that may have come in contact with the material and report to the Occupational Medical Service.
4. Do not permit others to enter the room until the appropriate authorities determine the area is safe to enter.

- Follow the established procedures for the decontamination and safe movement of scientific and medical equipment (NIH Instruction and Information Memorandum DL 90-2)

- Assist support personnel who may have to enter laboratories by removing hazardous materials from equipment/facilities to be serviced, forewarning them of the need for protective equipment or work practices, etc. When needed, provide the appropriate personal protective equipment.

Suggested Guidelines on the Safe Storage of Hazardous Chemicals in a Biomedical Research Laboratory

The use of chemicals is an essential part of modern biomedical science. Fortunately, most of the chemicals commonly used in the laboratory are not especially hazardous--but there are clearly exceptions.

In the laboratory, hazardous chemicals can be divided into four general categories--corrosives, flammables, reactives and toxics. In most cases, it is the immediate or obvious hazard that determines which category a particular chemical is classified.

The costs associated with the proper disposal of chemical wastes and the safe storage of chemicals in the research laboratory are inextricably linked. According to the American Chemical Society, the costs associated with chemical disposal are an **average of ten times** the purchase price. In some cases, there are no acceptable waste disposal options.

Controlling the increasing costs of proper chemical waste disposal and the inherent hazards of storing and working with hazardous chemicals requires re-thinking many of the ways that we purchase, handle and store laboratory chemicals.

General Principles

- Only purchase what you can reasonably expect to use during the next six(6) months.

- *Less is better. . .* Purchase containers in the smallest practical size. Whereas the per amount cost may be greater--significant savings are realized in reduced disposal costs and safer storage.

- When possible, buy what you specifically need. It is often possible to buy pre-made molar and normal solutions, thereby reducing the likelihood of waste.

- Glass breaks. When available, purchase chemicals in plastic containers. If this is not possible, purchase shatter resistant plastic coated bottles.

- Read labels. Most of what you will need to handle and store the chemical is found on the manufacturers' label.

- Rotate your chemical inventory. Indicate the date received and the date opened. Pay particular attention to expiration dates.

- Keep all chemical containers off of floors, carts and electrical equipment.

- Physically segregate your chemicals into their respective hazard categories--corrosive, flammable, reactive and toxic.

- Label the secondary storage containers or areas in which particularly hazardous chemicals may be used. These substances must be kept in a designated area.

- Relocate corrosive, flammable and reactive chemicals to locations below eye level. This simple task greatly reduces the likelihood of something falling from above and breaking.

- Cabinets with doors are safer locations than open shelves for hazardous chemicals.

- Convenient, impact resistant bottle carrier are available from the NIH Self-Service Stores.

    * The four liter carrier holds larger bottles safely (#6640-00-L01-1343).

    * The pint bottle carrier holds six one-pint bottles inside a domed lid with an easy to carry handle (6640-00-L01-1340).

- Avoid placing **any** chemical container in direct sunlight, underneath a sink or near heat sources.

- Never place volatile or flammable chemicals in unapproved refrigerators.

- Be especially careful with reactive chemicals. Obtain and read the Material Safety Data Sheet (MSDS) for each reactive chemical that you may have.

- The identification and disposal of unlabeled chemical containers is very expensive. Please label all containers in the laboratory with the following information.

    * The name of the chemical or stock solution.
    * The date made.
    * Your initials.

- Use and manage your chemical fume hood, wisely. Too many chemical containers or equipment can occlude the air slots and compromise the safety of the hood.

- Store all flammable chemicals in an approved flammable storage cabinet. If you do not already have a flammable storage cabinet, call your ICD Safety and Health Specialist for assistance (6-2346). Flammable storage cabinets come in all sizes.

- Follow the disposal guidelines provided by the NIH Waste Calendar.

Additional information on the safe storage of laboratory chemicals and a guide to chemical incompatability is available from your ICD Safety and Health Specialist by calling 6-2346.

VI Determination and Control of Exposures to Hazardous Chemicals

Hazardous chemicals may only be used in laboratory facilities specifically designed and engineered for such work. They may not be used in areas not intended for such use; including (but not limited to), offices, storage rooms, shared equipment areas, cold rooms and other areas lacking the appropriate facilities and a proper means of ventilation.

Local exhaust ventilation systems such as fume hoods and glove boxes are the preferred and primary method of controlling exposures to hazardous chemicals in the laboratory.

Assuming that fume hoods and other appropriate methods of containment are used properly and whenever needed; safe work practices are followed judiciously, and all laboratory and support personnel practice good personal hygiene-- the need for regular monitoring of airborne contaminates in the laboratory is unnecessary and impractical.

When a concern does arise over potential exposure to a laboratory chemical, specialized monitoring and chemical exposure determination is available from the industrial hygienists of the OSHB. Contact your ICD Safety and Health Specialist at 496-2346 to assist you.

As required by the laboratory standard, exposures to OSHA regulated chemicals in the laboratory must not *exceed* the Permissible Exposure Limits (PELs) or the recommended Threshold Limit Values (TLVs) when there is no PEL.

Special Note on Pregnancy

Personnel who are pregnant or considering becoming pregnant may have special concerns about working with chemicals that have potential reproductive hazards. Such concerns can be discussed with their supervisor, OSHB ICD Safety and Health Specialist (496-2346), or Occupational Medical Service (OMS) at 496-4411.

VII Personal Protective Equipment

Personal protective equipment (PPE) is a vital and *secondary* means of worker protection to be used in conjunction with *primary* means of control such as fume hoods.

Information and guidance on the selection and use of personal protective equipment is provided by your OSHB ICD Safety and Health Specialist (496-2346) and as part of the Laboratory Safety at the National Institutes of Health training program.

Various types of protective equipment; including chemically resistant gloves, aprons, eye and face protection, bottle carriers, etc., are available from the NIH self service store(s)-- consult the NIH supply catalog for more information or call your ICD Safety and Health Specialist for a updated list (496-2346).

Prescription safety glasses can be obtained from the Occupational Medical Service (496-4411).

Respirators *must not* be used in the laboratory without prior approval of the OSHB. Laboratory supervisors *are not* authorized to select or recommend the use respiratory protection, *regardless of the type*. Call your OSHB ICD Safety and Health Specialist if you feel that you may need respiratory protection. (The selection, fitting and use of respirators is referenced in OSHA standard 29 CFR 1910.134.)

VIII Precautions to be Taken When Working With Particularly Hazardous Substances

These substances include 'select' carcinogens, reproductive toxins and chemicals that have a high degree of acute toxicity. Substance specific information is available through the Technical Assistance Section of the OSHB (496-3353).

The laboratory supervisor is responsible for ensuring that appropriate additional precautions are taken when working with such chemicals.

Safe Work Practices With Particularly Hazardous Substances

- Maintain an accurate record of who uses these substances and the amounts used and stored in the laboratory.

- In the event that toxicologically significant quantities of a particularly hazardous substance are used on a routine or frequent basis, contact the Occupational Medical Service at 496-4411 to determine if medical surveillance may be warranted.

- Particularly hazardous substances must be kept in a secondary container to help prevent breaks and spills. This secondary container should be opened inside a chemical fumehood.

- A suitable label should be attached to this secondary container to alert others of the chemical contained therein and (if required) the need for special precautions. *For example:*_Warning-- Cancer Hazard_" or "_Highly Toxic_".

- Access to the laboratory should be controlled by the use of appropriate signs that warn of the hazards and indicate the precautions or approvals for entry.

- Additional containment devices (such as shielding or protective filters) may be needed to safely handle, store or protect equipment when using these chemicals.

- In addition to the use of the proper gloves, eye/face protection, other protective apparel or equipment may be needed. *Examples could include:* impervious gowns, aprons, gauntlets or other specialized protective equipment.

- Work surfaces should be protected from contamination through the use of disposable, absorbent, plastic backed paper. Replace contaminated paper as necessary and handle as hazardous waste.

- Specialized waste disposal may be needed. Contact the Environmental Protection

Branch at 496-7990 for assistance.

- On completion of work and before leaving the laboratory, remove all protective apparel and thoroughly wash hands, forearms, face, and neck.

IX Prior Approval for Work with Certain Hazardous Chemicals

Prior approval is warranted when working with certain hazardous chemicals and where there is a significant risk of exposure. This oversight process is followed when the proposed work meets one or more of the following criteria.

- The chemical(s) to be used can cause severe acute or lethal effects upon exposure by any route to quantities of 50 ug/kg or less.
- Highly unstable compounds or compounds that, if combined with other compounds in the procedure, will explode, are used.
- Compounds are used that may undergo chemical or physical changes during routine use, and generate by-products that may overcome standard control measures or penetrate available personal protective equipment to cause severe acute or lethal injuries.
- Specific chemicals and operations are used that have been determined by the Division of Safety to require approval above the level of the laboratory supervisor.

When one or more of the aforementioned criteria is met, the project principal investigator must ensure that:

A specific written safety protocol is developed and submitted to your OSHB ICD Safety and Health Specialist *for review prior to beginning work*. This safety protocol should include: (1) a thorough description of the chemical(s) used--including the potential physical and health effects, (2) a step-by-step review of the work to be performed, (3) a list of the required engineering controls and personal protective equipment, (4) where the chemical(s) are to be kept and proper labeling, (5) provisions for proper waste disposal and (6) decontamination procedures.

Evidence of employee training on the safety protocol is provided to the OSHB. This information must include a review of the safety protocol developed above, expected behaviors in the event of an emergency, the date the training was provided, and the name(s) and last four (4) digits of the social security number(s) of those trained. Assistance in laboratory safety training is available from the OSHB Safety Training Officer at 402-3535.

The proposed activities are conducted by specifically trained personnel in accordance with the approved protocol procedure.

Should the PI and the OSHB fail to resolve a significant issue regarding a protocol, then the matter will be referred to the ICD Scientific Director and/or the NIH Occupational Safety and Health Committee, as appropriate.

X Chemical Hygiene Plan Evaluation and Recordkeeping

The NIH Chemical Hygiene Plan will be evaluated and updated as needed by the Division of Safety and the NIH Occupational Safety and Health Committee. Comments and suggestions on the improvement of this document should be directed to the Chief, OSHB (496-2960).

Training and chemical exposure monitoring records are maintained by the OSHB. Individuals may request their records by contacting the Chief, OSHB (496-2960).

XI Services Provided by the NIH Division of Safety.

The NIH Division of Safety conducts a comprehensive testing and certification program for equipment such as chemical fume hoods, other local exhaust systems, biological safety cabinets, fire extinguishers and other safety related equipment.

Concerns about the proper selection, use, and functioning of protective equipment should be directed to your OSHB ICD Safety and Health Specialist.

Troubleshooting Guide for Chemical Fume Hoods

- o Call DES Building Maintenance when the air flow is low, the light is out, the sash is not working properly or the glass in the sash is broken.
- o Call OSHB Safety Support Section (496-3457) when a new hood needs certification or a hood has failed and needs recertification after repairs.
- o Call OSHB Safety Operations Section (496-2346) when a clearance is needed to remove a hood, to make repairs or (on some occasions) to replace a light bulb.

The Division of Engineering Services (DES) is responsible for the maintenance of NIH laboratory facilities. Problems related to laboratory facilities such as room temperature, plumbing, fixtures, repairs, etc. should be directed to the DES.

Call the appropriate number(s) listed in the yellow pages of the NIH telephone directory under 'Building and Engineering Services' or 'Leased Areas: Building Services'.

Medical Consultations

The NIH Occupational Medical Service, DS provides NIH employees the following services:

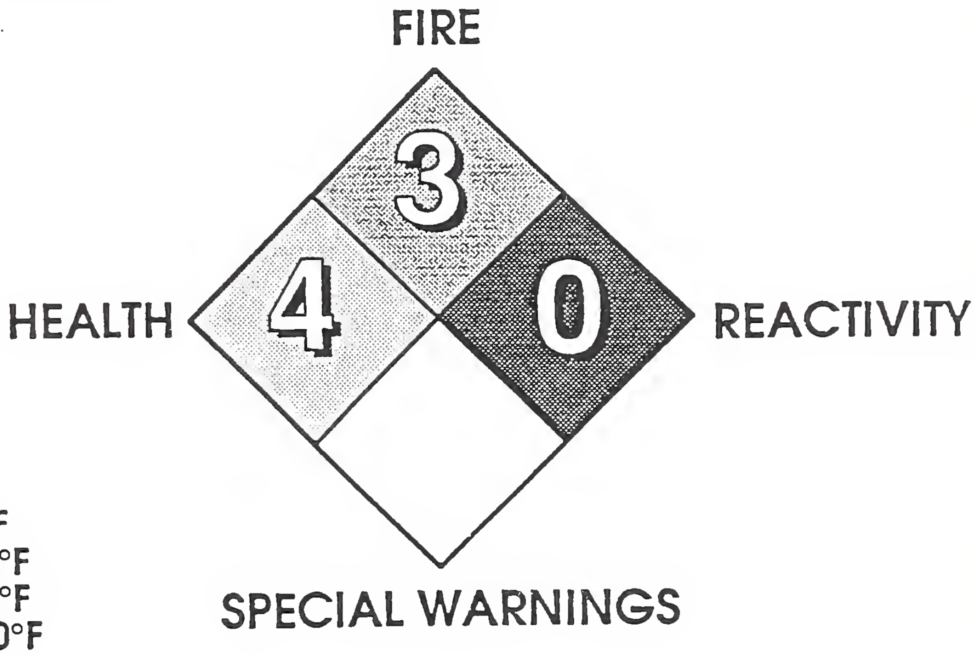
- Emergency treatment in the event of a spill or other unusual event results in an acute chemical exposure.
- Evaluation of an employee who develops signs or symptoms associated with possible exposures to a chemical used in the laboratory.
- Periodic medical surveillance when chemical exposure monitoring data reveal an exposure at or above the action level for an OSHA regulated substance.
- Maintains medical records.

In the Event of a Medical Emergency

- Dial 116 (on campus) to summon an ambulance.
- Dial 9-911 (off campus) to summon local emergency response services.

National Fire Protection Association (NFPA) Hazard Warning Labeling System

Health Hazards : Blue Diamond
Fire Hazards : Red Diamond
Reactivity : Yellow Diamond
Special Warning : White Diamond



HEALTH HAZARDS

4 - Extreme
3 - Serious
2 - Hazardous
1 - Slight
0 - Non-Hazardous

FIRE HAZARDS

4 - Flash Point Below 73°F
3 - Flash Point Below 100°F
2 - Flash Point Below 200°F
1 - Flash Point *Above* 200°F
0 - Will Not Burn

REACTIVITY HAZARDS

4 - Extreme: May Detonate
3 - Serious: Heat/Shock May Detonate
2 - Moderate: May Undergo Violent Reaction
1 - Potential: Unstable if Heated
0 - Stable: Under Most Conditions

SPECIAL WARNINGS

Oxidizer : OXY
Acid : ACID
Alkali : ALK
Corrosive : COR
No Water : ₩
Radiation : ☢

Essential Information On The Establishment of A "Designated Area" in the Laboratory

The Occupational Safety and Health Administration (OSHA) has issued a standard entitled, "Occupational Exposure to Hazardous Chemicals in Laboratories" (29 CFR 1910.1450).

Under this standard, chemical substances of moderate to high chronic toxicity or of high acute toxicity must be properly stored in a "Designated Area".

A "Designated Area" is a specified and well labeled area in which carcinogens, teratogens, embryotoxins, or other chemicals with significant acute or chronic toxicity are used or kept in the laboratory. A "Designated Area" could be a cabinet, desiccator, fume hood, refrigerator or other appropriate area where toxic substances can be stored or used.

An approved sign should be used to alert other laboratory personnel that such substances are present. It also serves to alert housekeeping and maintenance personnel that they should be especially careful when working around these areas.

• IMPORTANT THINGS TO REMEMBER •

1) All chemicals should be segregated and stored according to their primary hazard category in separate areas to reduce the potential for cross-reaction.

2) Select corrosion resistant shelving for the storage of corrosive chemicals. Do not store acids with bases. Have immediate access to an eyewash and safety shower.

3) Reactive chemicals can be especially hazardous

4) Flammable chemicals present an immediate physical hazard because of the chance of fire. In addition, many flammable chemicals have associated health risks.

5) When in doubt, read the label and Material Safety Data Sheet for more information.

6) Select, obtain, and wear the appropriate protective equipment whenever you handle hazardous substances.

7) A working and uncluttered fume hood should be used whenever you work with a toxic substance

8) Practice good personal hygiene and promote frequent hand washing.

SAFETY NOTES

Eye Protection in the Laboratory

Approximately 1000 industry-related eye injuries occur in the United States every day. More than 900 of them could be prevented by the use of proper eye protection. Inadequate or nonexistent precautions can result in disabling eye injuries, which now account for an average of five percent, or 100,000, of all annual disabling work injuries in the U.S.

Biomedical research laboratories are breeding grounds for many kinds of potential eye injuries, and everyone working in them should be aware of these inherent dangers and how to circumvent them. A variety of eye protective devices are available to protect against these unique hazards. Proper eye protection is required for all persons entering sites where chemicals are manipulated, where impact injuries may occur, where exposure to ultraviolet (UV) transilluminators or lasers is possible, or where eye exposure to infectious materials is a risk.

Splash Hazards: Chemical splashes and vapors constitute the primary eye hazards in the laboratory, although persons working with infectious microorganisms, including those working with non-human primates, also may be at risk of ocular exposure. Protection of the facial mucous membranes from splashes is best accomplished by wearing a combination of mask and goggles or a face shield. The following safety devices may be used to protect against splashes of hazardous chemicals or biological agents.

Face-shields, which come in a variety of sizes, thicknesses, materials, lengths and widths (and which can be selected to meet individual requirements) may be the best form of protection against splashes. General-purpose face-shields are available through the NIH self service stores. Upper and lower shells can be added to face-shields to help protect the face from hazards from above and below.

Goggles are another good form of protection from splashes; some also protect against vapors. However, goggles made with vents to allow moisture to escape provide less protection. Some goggles are available through the self service stores.

Eye Impact Hazards: Eye impact injuries are another hazard commonly posed both by laboratory and non-laboratory conditions and materials. Pressurized containers, vaccum containers, chemical reactions, power tools, and human error can transform solid and frequently sharp objects into dangerous projectiles, or eye impact hazards.

Safety glasses are designed to protect the eyes from impact hazards and may also provide some protection from a direct splatter but will not protect against vapors or splashes to the side of the face. NIH employees who have a need to protect their eyes from impact hazards may obtain safety glasses through the Occupational Medical Service (use NIH Form 29-2, Request for Safety Glasses).

Safety glasses, which can be ground to meet your corrective prescription, are made with tempered glass and have special features to resist direct impact; they also can be equipped with side shields. Safety glasses should be worn in areas where eye impact injuries can occur. If normal prescription glasses or contact lenses are worn in these areas, they should be covered by goggles or face-shields.

Goggles and face-shields are also recommended personal protective devices against possible eye impact injuries. Full face shields will provide excellent protection from flying objects, such as shattered glass or metal shards.

Another way to prevent eye impact injuries is to cover or surround the potential source of the problem with a shield. This type of physical barrier has several advantages: it can be left in

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place at all times; will not be uncomfortable or forgotten like glasses, goggles, or face-shields; and protects everyone in the vicinity. Source shields should be removed only when necessary and always replaced immediately.

Radiant & Radioactive Hazards: Specific safety glasses, goggles, face shields and source shields can prevent the serious and lasting eye damage induced rapidly by exposure to UV or laser lights. The appropriate type of goggles for protection against laser light is determined by the exact wavelength and power or laser source material being used. The manufacturer of the equipment or your occupational safety and health consultant can assist you in making that decision. UV eye and face protection is provided by face- and source-shields that effectively absorb UV light below 400 nanometers wavelength while still allowing the wearer to see clearly.

Transparent source-shields also may be used to protect the eyes and the rest of the body from radioactive sources. The Radiation Safety Branch health physicists can advise you on selecting the proper shields. If a source-shield is to be used inside a Class II Biological safety cabinet, seek advice from your occupational safety and health consultant on selecting an appropriate shield so that the air flow inside the cabinet is not compromised.

Contact Lenses: Laboratory personnel who wear contact lenses are at unique risk of serious eye damage. Soft lenses may absorb chemicals and become a source of introducing dangerous materials to the eye. Splashed chemicals or even vapors and gases can become trapped behind contact lenses; serious damage may occur before the lenses can be removed and the eyes flushed. With the lens in place, it is impossible to effectively irrigate the eye, thus increasing the injury to it. Lens removal may require anesthesia to counteract the uncontrollable clamping together of the eyelids that can follow a painful chemical splash to the eye. Given the pain and fear that follow such a splash, the presence of contact lenses may even be forgotten and cause more problems.

Laboratory workers who elect to wear contact lenses in laboratory situations should also wear auxiliary eye protection, such as goggles or a face shield.

Eyewash Stations & Procedures: Accidents do happen. When chemicals are splashed into your eyes or when your eyes are irritated by vapors, flush them immediately. To properly flush out a chemical, hold the eye(s) open and irrigate with copious amount of water for at least 15 minutes. Regardless of how minor an injury may seem, medical attention should be sought immediately thereafter from the Occupational Medical Service (OMS).

In the same way that proper safety devices can prevent injuries, good emergency-response equipment can help to stem or ameliorate damage when accidents do occur. Eyewash stations—which an Occupational Safety and Health Administration regulation states must be available where corrosive materials are used—are a case in point. Accessible to many NIH laboratories, they provide the necessary ingredients for a thorough eye cleansing: a low pressure spray of comfortable-temperature potable water for at least 15 minutes. The very limited water supply in portable eyewash bottles precludes their effectiveness and therefore are not recommended.

Eye wash stations can become a growth site for free-living amoebae and other microorganisms that can cause eye infections. This minor risk can be greatly reduced by flushing out the station for at least three minutes every week.

All workers in the lab should be aware of the location of the nearest eyewash station. It is recommended that the selection of an appropriate eyewash station and its location be discussed with your ICD occupational safety and health consultant. Call 496-2346 for more information.

Good vision is very important! Hazard awareness and use of eye safeguards just make good sense.

For Assistance with	Contact	Telephone
Advice on selection of proper eye protection	ICD occupational safety & health consultant	496-2346
Ordering safety glasses	Occupational Medical Service (Bldg. 10, 6C303; 8am-5pm, M-F)	496-4411
Radiation safety issues	ICD health physicist	496-5774
Face-shields, goggles	Self service stores	
	Building 10	496-2051
	Building 31	496-4430
	Building 35	496-4095

USE OF ACRYLAMIDE IN GEL ELECTROPHORESIS

(Synonyms: 2-propenamide; acrylic acid amide; propenoic acid amide)

BACKGROUND

Acrylamide is a chemical widely used in molecular biology for making various types of gel electrophoresis. Owing to its widespread use in biomedical research and its potential toxicity, questions about the hazards associated with the use of acrylamide are common.

CHEMICAL & PHYSICAL PROPERTIES

Acrylamide is a colorless-to-white, odorless, flake-like crystal. It is highly soluble in water and soluble in ethanol, acetone and ether. It melts at 84.5°C and its boiling point is 125° C (25 mm Hg). It has a very low vapor pressure (0.007 mm Hg at 25° C). Acrylamide is stable at room temperature, but it is quite reactive and known to polymerize violently when heated to the melting point or placed under UV light. The solid should be stored in a cool, dark place, away from strong oxidizers.

ADVERSE HEALTH EFFECTS

Acrylamide is a skin and respiratory tract irritant. Prolonged exposures may result in neurotoxicity. The cumulative nature of acrylamide neurotoxicity dictates that skin exposure be minimized.

Acute Exposure

o Skin Exposure

Skin splashes may cause burns or local irritation. Prolonged skin contact results in local dermatitis and rash. Acute allergic contact dermatitis from acrylamide used for preparation of polyacrylamide gels has been reported.

o Eye Exposure

Acrylamide solutions are strong eye irritants and may produce conjunctival irritation and corneal injury.

o Respiratory Exposure

Inhalation of acrylamide vapors may cause dizziness or suffocation. Report to the Occupational Medical Service (OMS) immediately for medical assistance. Dial 116 for an ambulance if needed.

Cumulative Exposure

Acrylamide is a cumulative neurotoxin. Prolonged dermal exposure may cause progressive peripheral neuropathy. However, chronic diseases related to its exposure have not been reported in the biomedical research worker population, so far.

Peripheral neuropathy is the common form of neurotoxicity seen in humans. A characteristic contact dermatitis of the palms often precedes the neurologic syndrome. Excessive sweating of the feet and hands may also occur.

Reproductive Effects

Currently, there is no information on possible reproductive effects of acrylamide in humans.

In animal studies acrylamide crosses the placenta and causes both experimental reproductive loss and neurotoxic effects in neonates.

Mutagenicity

Mutagenic tests have shown conflicting results. Tests for mutagenicity have been negative in bacteria but positive in both mouse germ and somatic cells.

Carcinogenicity

Acrylamide is a possible human carcinogen. There is sufficient evidence of carcinogenicity in animals. However, there are inadequate data for deciding whether this also applies to humans.

FIRST AID

Skin & Eye Exposure

For skin exposure, remove

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contaminated clothing and wash skin with soap and water. For eye exposure, irrigate at once with copious amounts of running water for at least 15 minutes while holding eyelids opened.

In case of an exposure or injury report to OMS for medical assistance (496-4411; see location below). For an ambulance, call the NIH Fire Department at 116 (off campus, 9-911).

SAFETY PRECAUTIONS

Reduce Supply

Minimize the purchase of acrylamide. Buy only the amount expected to be used within a few months. This will decrease the amount of space needed to store excess chemicals. When possible, order bulk chemicals in the smallest container available. This will make it easier to handle, minimize the amount subject to spillage and save on cost for waste disposal of the excess. Since acrylamide is hygroscopic, keep container tightly closed,

Weigh Acrylamide Carefully

Weigh acrylamide in tared containers, e.g., "weigh boats", to reduce the risk of exposure. Do not shake the container. Use a spatula to lift the chemical out of the container. To transport the weighed acrylamide, place the tared containers inside a covered tray.

Use Chemical Fume Hood

To reduce skin and inhalation exposure, use a chemical fume hood or ventilated glove box with a dedicated balance. Powder crystalline flakes may remain airborne for a long time. Use a wet paper towel to wipe the contaminated areas.

To prevent exposure to aerosols and eye splashes, mix buffers inside a chemical fume hood with the sash lowered to the appropriate level indicated by the certification label.

Buy Pre-made Solutions

Commercially prepared solutions for making polyacrylamide gels are easier to handle than powdered acrylamide and are relatively non-toxic.

Wear Personal Protective Gear

Wear disposable polyvinyl chloride (PVC) or latex gloves to reduce skin exposure. Gloves should be changed regularly. Dispose of contaminated and used gloves in Medical Pathological Waste (MPW) burn boxes.

Wear a lab coat and safety glasses to protect yourself in the event of a chemical spill or eye splash. Do not wear sandals or shorts while handling potentially hazardous chemicals to minimize the potential for skin exposure.

GEL WASTE DISPOSAL

If gel waste is not radioactive, place it in a labeled, plastic-lined cardboard box and dispose of it as hazardous chemical waste.

CHEMICAL SPILL

If acrylamide is spilled, do not attempt to clean it up yourself. Evacuate the area and call the NIH Fire Department to clean up the spill.

REFERENCE

Ducatman, AM; Liberman, DF, Worker Hazards in the Biotechnical Industry, in *Hazardous Materials Toxicology*, Sullivan and Krieger (Eds), Williams & Wilkins, 1992.

For Assistance with	Contact	Telephone
Safety Questions	ICD Occupational Safety and Health Specialist	496-2346
Waste Disposal	Environmental Protection Branch	496-7990
Chemical Waste Pickup	Chemical Waste Contractor	496-4710
Medical Assistance	Occupational Medical Service (Bldg 10/6C303, 7 a.m. to 10 p.m., M-F)	496-4411
Emergency Response; Spills, Ambulance	NIH Fire Department (Off Campus)	116 (9-911)

SAFE USE OF CHLOROFORM IN THE BIOMEDICAL LABORATORY

Synonyms: Trichloromethane, Formyl Trichloride, Methane Trichloride

BACKGROUND

Chloroform is a halogenated solvent that is frequently used in biomedical laboratories. In molecular biological procedures, chloroform, along with mixtures of phenol and isoamyl alcohol, is used to extract proteins. Proteins are denatured and collect in the organic phase or at the interphase, while nucleic acids remain in the aqueous phase.

CHEMICAL AND PHYSICAL PROPERTIES

Chloroform is a heavy, clear, colorless, volatile, nonflammable liquid with a pleasant, non-irritating, characteristic, sweet odor. It has a slight sweet taste. It is slightly soluble in water, but it is miscible with alcohol, organic solvents, or oils. An odor threshold of 85 ppm has been reported for chloroform.

ADVERSE HEALTH EFFECTS

Chloroform is a central nervous system (CNS) depressant that is also toxic to the liver and kidneys. It is a potent general anesthetic; however, its toxicity to the liver and kidneys makes it unsuitable for use in humans. Inhalation of high concentrations have resulted in fatalities. The main routes of exposure in the biomedical laboratory are inhalation, dermal, and ocular contact.

Inhalation Exposure

Depending on the dose and exposure duration, chloroform may cause headache, fatigue, dizziness, respiratory depression, chemical pneumonitis, pulmonary edema, metabolic acidosis, CNS depression, cardiac arrhythmias, and acute chloroform-induced hepatotoxicity.

Symptoms of lassitude, digestive disturbances, and mental dullness were reported in a group working in 20-70 ppm chloroform; these symptoms were more severe for workers exposed to 80-240 ppm. Exposure to 900 ppm for a short time causes tiredness, dizziness, and headache.

No studies were located regarding musculoskeletal, dermal/ocular, immunological, reproductive, genotoxic, or carcinogenic effects in humans after inhalation exposure to chloroform.

Dermal Exposure

Chloroform may produce chronic irritation as a result of its defatting properties. It is not known to produce skin sensitization.

No studies were located regarding respiratory, cardiovascular, gastrointestinal, musculoskeletal, hematological, hepatic, renal, immunological, neurological, developmental, reproductive, genotoxic, or carcinogenic effects in humans from skin exposure to chloroform.

Eye Exposure

Chloroform vapor can cause conjunctival irritation and blepharospasm (twitching of the eyelids). Burning pain and corneal epithelium injury may occur from chloroform liquid splashed in the eye. Complete spontaneous recovery is the usual course following appropriate care.

Oral Exposure

Information regarding mortality in humans after oral exposure to chloroform is limited. Retrosternal soreness, pain on swallowing, and gastric distress with vomiting have been reported in cases of ingestion of chloroform. The liver and kidneys are the primary targets of chloroform toxicity. Jaundice, liver enlargement, and tenderness may develop in patients following chloroform ingestion. Renal effects include oliguria, increased blood urea nitrogen and creatinine levels, as well as albuminuria and casts in the urine.

Carcinogenicity

Chloroform is classified as a suspect or potential human carcinogen. The data are insufficient to support any conclusion regarding the carcinogenic potential of chloroform in humans. There is sufficient evidence that chloroform administered orally is carcinogenic in mice and rats.

Reproductive Effects

In humans it is known that chloroform crosses the placenta; however, there is no evidence that reproductive toxicity is attributable to this agent in the absence of maternal toxicity. In experimental animals, chloroform is not a strong teratogen, but it is embryotoxic.

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FIRST AID

Inhalation Exposure

Move victim to fresh air while avoiding self-exposure to chloroform. Report to the Occupational Medical Services (OMS) for medical evaluation. If transportation or assistance is needed, contact NIH Fire Department (on campus, dial 116). If off campus, dial 9-911.

Eye and Dermal Exposures

If an eye exposure occurs, flush the eye with water immediately for at least 15 minutes. Call the NIH Fire Department for assistance and transportation to OMS or Suburban Hospital.

For skin exposure, flush the chloroform off with water, and wash exposed areas thoroughly. Remove and isolate contaminated clothing as soon as possible. Report to OMS as soon as possible for medical evaluation.

Ingestion Exposure

Do not induce vomiting. Call NIH Fire Department for ambulance transportation (on campus, dial 116) to OMS or Suburban Hospital as soon as possible. If off campus, dial 9-911.

SAFETY RECOMMENDATIONS

The current permissible exposure limit (PEL) set by the Occupational Safety and Health Administration (OSHA) for chloroform in the workplace is 2 ppm (averaged over an 8-hour workday, for a 40 hour workweek). Be aware that this PEL is approximately 40 times less than the odor threshold of chloroform in the air.

Personal Protective Equipment

Work in a chemical fume hood or other well-ventilated area. Wear a buttoned lab coat, long pants, and closed toe and heel shoes to prevent skin exposure.

Wear N-Dex disposable nitrile gloves for handling glassware and other equipment that contain chloroform. The N-Dex disposable nitrile gloves, as well as most gloves commonly used in the laboratory, provide limited protection because chloroform rapidly penetrates glove materials. Therefore, gloves should be changed frequently, especially after overt contamination. Remove contaminated gloves immediately to prevent permeation breakthrough exposure.

Wear safety glasses when handling small (microfuge) amounts of chloroform. Wear safety goggles when dispensing from original containers or large volume, bulk solutions. If a face splash is a possibility, wear a face shield.

Storage

Avoid storing chloroform with strong oxidizers. Chloroform emits phosgene and chlorine when oxidized by strong oxidizers. Keep chloroform away from heat sources. When heated to decomposition, chloroform emits toxic and corrosive fumes.

Chemical Waste Disposal

Collect used or excess chloroform and dispose of it as chemical waste in its original container (print "WASTE" and discard date on label). If the original label is loose or illegible, complete and attach an NIH Chemical Waste Tag. If a container other than the original is used, mark **XXX** through the label. Complete and attach an NIH Chemical Waste Tag to the container. Call the Chemical Disposal Service to obtain chemical waste containers.

Chemical Spill

For chemical spills on campus, call the NIH Fire Department (116) for assistance and cleanup. For spills occurring off campus, call the Montgomery County Emergency Services (9-911) and then the NIH Fire Department (496-2372).

REFERENCES

U.S. Department of Health and Human Services, Public Health Service, *Toxicological Profile for Chloroform*, Agency for Toxic Substances and Disease Registry, Atlanta, GA, April 1993.

American Conference of Governmental Industrial Hygienitsts, Inc., "Chloroform" in *Documentation of the Threshold Limit Values and Biological Exposure Indicies*, 6th Edition, ACGIH, Cincinnati, OH 1991.

For Assistance with	Contact	Telephone
Safety and Health Questions	ICD Occupational Safety and Health Specialist	496-2346
Chemical Waste Questions	Environmental Protection Branch	496-7990
Chemical Waste Pickup	Chemical Waste Contractor	496-4710
Medical Assistance	Occupational Medical Service (Bldg 10/6C303, 7 a.m. to 10 p.m., M-F)	496-4411
Emergency Response; Spills, Ambulance	NIH Fire Department (Off Campus)	116 (9-911)

USE OF FORMALDEHYDE IN BIOMEDICAL RESEARCH

BACKGROUND

Formaldehyde is often used in the biomedical laboratory as formalin (a 37% to 50% aqueous solution). It is used to preserve human and animal tissues, fix histological specimens, alter bacterial toxins to toxoids, as well as in procedures for agarose gel electrophoresis.

CHEMICAL AND PHYSICAL PROPERTIES

Formaldehyde is a pungent, colorless gas that is miscible with water and many organic solvents. Ordinarily methanol (0.5% to 15%) is added to formalin to inhibit polymerization. Formalin with methanol is a combustible liquid.

TOXICITY AND HEALTH EFFECTS

Formaldehyde acts primarily as an irritant. It is also a strong sensitizer which may elicit allergic reactions following subsequent exposures.

Main routes of exposure to formaldehyde in the biomedical laboratory are dermal contact and inhalation. Formaldehyde is readily absorbed from the respiratory and oral tracts and to a much lesser degree through the skin.

Inhalation Exposure

Formaldehyde is highly irritating to the upper respiratory tract, mucous membranes, and eyes. Acute inhalation may cause bronchitis, pulmonary edema, pneumonitis, pneumonia, and respiratory failure. Its odor threshold concentration is about 0.1 part per million (ppm). Concentrations as low as 0.5 ppm can cause eye, nose, and throat irritation. At 5 to 10 ppm, cough and tightness in the chest may appear. Depending on the exposure concentration, other symptoms may include: headache and nausea, wheezing, shortness of breath, rhinitis, laryngospasm, tracheitis, and anosmia (absence of the sense of smell). Anosmia may contribute to exposures of higher than acceptable levels.

Inhalation of 25 to 30 ppm may cause severe respiratory tract injury leading to pulmonary edema and pneumonitis. The severity of the injury increases as a function of the concentration and duration of exposure. Exposure to 100 ppm is immediately dangerous to life.

Bronchial asthma-like symptoms have been reported in certain individuals who inhaled formaldehyde. Few cases of respiratory sensitization in humans have been documented. However, formaldehyde seems to act more commonly as a direct airway irritant in persons who have bronchial asthma from other causes.

Dermal Contact

Formaldehyde solutions (greater than 2%) are irritating to the skin and may cause blanching, smarting, drying, cracking and scaling. Concentrated solutions can be corrosive. A person sensitized to formaldehyde may react with allergic eczematous dermatitis or hives.

Eye Exposure

Formaldehyde vapor is irritating to the eyes. Concentrations of 0.5 ppm may produce eye irritation and tearing; 5-10 ppm is extremely irritating. Formalin splashed in the eyes may cause injuries ranging from a minor, transient discomfort to severe, permanent corneal damage and loss of vision. The severity of damage to the eyes depends on the concentration and how rapidly the formalin is flushed from the eyes.

Carcinogenicity

The Occupational Safety and Health Administration (OSHA) classifies formaldehyde as a potential human carcinogen. Although chronic inhalation of formaldehyde by rats produced cancer of the nasal cavity, the evidence for it being a human carcinogen is limited. Exposures have been associated with slight increases in risks of cancers of the lung, nasopharynx, oropharynx, and nasal passages in humans.

Reproductive Effects

Human studies are limited, but one investigation into the effect of occupational exposure to formaldehyde in hospital workers did not demonstrate an increase in spontaneous abortion or birth defects in women exposed to formaldehyde before and during pregnancy.

FIRST AID PROCEDURES

Eye Exposure

Flush eyes immediately with large amounts of water for 15 minutes while lifting the lower and upper lids. Call the NIH Fire Department (on campus, dial 116) for transportation to Occupational Medical Service (OMS) or Suburban Hospital. If off campus, dial 9-911.

Dermal Exposure

Flush the formalin off the skin immediately and then wash with soap and water. If a worker's body is exposed to formaldehyde, an emergency shower should be used

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for quick drenching of the body.

Inhalation Exposure

Remove the patient from further exposure. If symptoms persist, hospitalization may be required. Report to OMS (496-4411) as soon as possible for a medical evaluation. Call the NIH Fire Department (dial 116) for transportation to OMS or Suburban Hospital. For off campus assistance, dial 9-911.

SAFETY RECOMMENDATIONS

Personal Protection

Proper protective equipment and clothing should be used to reduce the risk of direct skin or eye contact with formaldehyde. This includes wearing: (1) a fully buttoned lab coat, long pants, and shoes with closed toes and heels; (2) N-Dex disposable nitrile gloves; (3) chemical splash goggles. Safety glasses provide only limited protection. A face shield should be worn when there is a chance of a chemical splash. These items are easily available at the NIH Self-Service Stores.

The storage, preparation, or consumption of food or beverages and the application of cosmetics are prohibited in all laboratories.

Stock Reduction

Limit the purchase of formalin to the amount expected to be used within a few months. This will decrease storage space requirements. Order bulk stock solutions in smaller containers to minimize the amount subject to spillage and to save on costs of disposing excess chemicals. In addition, consider replacing formalin for preserving tissue specimens with a less hazardous product.

Work Area Safety Equipment

Work should be conducted in a chemical fume hood or a well-ventilated area. A safety shower and an eye-wash fountain should be easily accessible. Test the eye-wash fountain every two weeks to ensure its adequate operation. Safety showers are tested by maintenance personnel only.

Storage

Keep container tightly closed. Store in a cool, dry, well-ventilated area or in a flammables storage cabinet. Use collecting trays to retain any leakage. Separate from oxidizing and alkaline materials.

Disposal of Contaminated Materials

Contaminated clothing should be removed immediately and placed in a clear plastic bag (medium: NSN-8105-01-195-8730 and large: NSN-8105-00-826-6468). Close bag with either filament tape or bag closure tie. Place bag in a plain cardboard box and secure box with filament tape (**Do not** use an MPW box or orange autoclave bag). Complete and attach an NIH Chemical Waste Tag (NSN-7530-00-L07-5985) to the box. Call Chemical Disposal Service for pick-up and disposal (496-4710).

Chemical Waste Disposal

Collect used or excess formalin and dispose of it as chemical waste in its original container (print "WASTE" and discard date on label). If the original label is loose or illegible, complete and attach an NIH Chemical Waste Tag. If a container other than the original is used, mark **XXX** through the label. Complete and attach an NIH Chemical Waste Tag to the container. Call the Chemical Disposal Service to obtain chemical waste containers.

Chemical Spill

For chemical spills on campus, call the NIH Fire Department (116) for assistance and cleanup. For spills occurring off campus, call the Montgomery County Emergency Services (9-911) and then the NIH Fire Department (496-2372)

REFERENCES

NIOSH/OSHA, Occupational Health Guideline for Formaldehyde, in *Occupational Health Guidelines for Chemical Hazards*, F. Mackinson, *et al* (Eds), U.S. DHHS (NIOSH) Publication No. 81-123, 1988.

Clary, J.J. and Sullivan, Jr., J.B., Formaldehyde, in *Hazardous Materials Toxicology*, Sullivan and Krieger (Eds), Williams & Wilkins, pp 973-980, 1992.

American Conference of Governmental Industrial Hygienists, "Formaldehyde", in *Documentation of The Threshold Limit Values and Biological Exposure Indices, Vol II*, ACGIH, Inc., Cincinnati, Ohio, pp 664-688, 1991.

For Assistance with	Contact	Telephone
Safety and Health Questions	ICD Occupational Safety and Health Specialist	496-2346
Chemical Waste Questions	Environmental Protection Branch	496-7990
Chemical Waste Pickup	Chemical Waste Contractor	496-4710
Medical Assistance	Occupational Medical Service (Bldg 10/6C303, 7 a.m. to 10 p.m., M-F)	496-4411
Emergency Response; Spills, Ambulance	NIH Fire Department (Off Campus)	116 (9-911)

USE OF HYDROGEN FLUORIDE IN PROTEIN SYNTHESIS

(Synonyms: Hydrofluoric Acid, HF)

BACKGROUND

Investigators working in the area of protein synthesis often use HF to cleave covalently bonded peptides from commercially prepared peptide-resins.

In general, the procedure uses a HF-reaction apparatus (Peninsula Labs*) where anhydrous HF is transferred from the compressed gas cylinder into a teflon holding cylinder. Then it is transferred into a teflon reaction cylinder containing the peptide-resin. After the reaction is completed, the HF is transferred into an absorption cylinder with the aid of a vacuum pump, where it is neutralized.

CHEMICAL & PHYSICAL PROPERTIES

HF is one of the most chemically active substances known. It exists as a colorless gas above its boiling point (19.5^{0} C). HF is a fuming liquid at room temperature,

Anhydrous HF is highly corrosive and reacts with water or steam to produce toxic and corrosive vapor. HF attacks glass, ceramic materials and some forms of plastics, rubber, and coatings.

ACUTE EXPOSURE

Skin Exposure

HF is an extremely toxic chemical. It rapidly penetrates skin and deep tissues. HF burns are characterized by progressive tissue necrosis and severe pain. Exposure to concentrations above 50% may be felt within minutes, if not immediately. Exposures below 50%are more variable in their presentation. Onset of pain may be delayed for 2 to 24 hours.

Eye Exposure

HF causes severe eye irritation and deep-seated burns. If HF is not removed immediately by flushing with water, blindness may result.

Respiratory Exposure

HF is a severe respiratory irritant. Its strong irritating odor may be detected at concentrations near the OSHA 8 hour permissible exposure limit (3 ppm) and serve as a warning sign of HF presence. Inhaled HF, especially from a splash or spill around the face, may cause lung congestion. Onset of pulmonary edema can be delayed for hours and may be rapidly fatal when it occurs.

FIRST AID

Dial 116 for transportation by ambulance or go directly to Occupational Medical Service (OMS) immediately. During off hours, go to the Suburban Hospital Emergency Room. If off campus, dial 9-911.

Skin: Flush skin with water at once and continue flushing for 5 minutes; then apply calcium gluconate gel. If gel is not available, wash 15 minutes. Remove contaminated clothing without delay.

Eye: Wash copiously with water for at least 15 minutes. Hold eyelids away from eye to increase eye exposure to the wash (Do not apply calcium gluconate gel to eye).

SAFETY PRECAUTIONS

o Calcium Gluconate Gel (2.5%), available from OMS, should be readily accessible.

o An emergency eye wash and shower must be easily accessible.

o An extra pair of heavy-duty nitrile gloves for person assisting the victim should be readily available.

o A telephone must be easily accessible to call for help.

USER PROTECTIVE EQUIPMENT

HF users are required to wear heavy- duty gloves (chemical resistant, nitrile gloves, Cat. no. 6515-00-L03-2441/2/3), splash proof safety goggles, and acid resistent aprons, as well as other appropriate protective equipment as needed, to help prevent exposure. These items are available at the NIH Self-Service Stores.

CHEMICAL FUME HOOD

o All work with HF must be conducted inside a certified chemical fume hood which is dedicated for use with this chemical only.

o Bottles and equipment which may obstruct air flow along the work surface should be removed or placed on a shelf.

o The level of the sash should be lowered to the height indicated on the hood certification sticker.

o All equipment must be placed at least 6 inches inside the fume hood.

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PROPER MAINTENANCE OF EQUIPMENT & HF GAS CYLINDER

HF Reaction Apparatus

o Rubber tubing, seals and washers can become brittle with use. They should be properly maintained to prevent leakage. All tube fitting joints should be tightly clamped.

o To detect HF leakage, a commercially available manometer made for this purpose can be attached to the HF-reaction apparatus.

o Leaks can also be detected with litmus paper. First, dampen the paper with water. Then pick up the damp litmus paper with a pair of tongs and place it near fitted areas and places where leaks may occur. The paper will change color upon contact with acidic HF vapors.

HF Gas Cylinder

o The HF gas cylinder must be stored upright and securely restrained at all times to prevent it from toppling over. Keep it away from heat. Ensure connections are secured and check for leaks.

o Wear protective clothing and equipment when connecting, opening or disconnecting valve. Do not use cylinder valve for control; connect it to a specially designed regulator. A nickel-cadmium tank regulator valve is recommended for HF tanks. Point the regulator spout toward the rear of the hood. Label opening date on tank.

HF Absorption Cylinder

o This cylinder contains anhydrous calcium oxide (CaO) for neutralizing unreacted HF. As CaO is used up, the upper portion of the cylinder gradually becomes hotter than the lower portion. CaO is completely exhausted when no additional heat is generated when HF is pumped through the cylinder. For heavy users, two cylinders connected in series are recommended.

o Also, CaO expands when it is hydrated. When it presses against the glass porthole on top of the cylinder, the CaO should be replaced. Label replacement date on the cylinder.

Vacuum Pump

The vacuum pump must be placed inside the fume hood with its exhaust directed toward the rear exhaust slot.

Decontamination

Tools and equipment used on HF system should be neutralized with 10% Na_2CO_3 solution, washed, dried and inspected after each use or before repair. Verify completion of neutralization with indicator paper.

SAFETY TRAINING

Persons using HF must know its hazards, symptoms of exposure, first aid, emergency procedures, and precautions. They must be trained thoroughly on the proper use of the equipment, including its operation, replacing parts, bleeding lines, testing for leakages, handling compressed gas cylinders and have reviewed the approved standard operating procedure for the experiment. Consult your ICD Safety and Health Consultant for safety information.

NIOSH LABELING REQUIREMENTS

Anhydrous HF containers shall bear the following label:

DANGER!
HYDROGEN FLUORIDE, ANHYDROUS
EXTREMELY HAZARDOUS LIQUID & GAS
SEVERE INHALATION, SKIN, AND EYE HAZARD, CAUSES BURNS.
DO NOT BREATHE GAS.
DO NOT GET IN EYES, ON SKIN, ON CLOTHING

Post warning signs on room entrances.

REFERENCES

NIOSH Recommended Standard for Occupational Exposure to Hydrogen Fluoride, CDC, National Institute for Occupational Safety and Health, U.S. Government Printing Office Publication No. 1979-657-012/318.

Krenzelok, E.P., Hydrofluoric Acid, in *Hazardous Materials Toxicology,* Editors: Sullivan, J.B., Jr. and Krieger, G.R., Williams and Wilkins Publishers, 1992, pp 785-790.

Peninsula Laboratories, Inc., 1992-93 Catalog, 611 Taylor Way, Belmont, California 94002-4041

For Assistance with	Contact	Telephone
Safety Questions	ICD Occupational Safety and Health Consultant	496-2346
Waste Disposal	Environmental Protection Branch	496-7990
Chemical Waste Pickup	Chemical Waste Contractor	496-4710
Medical Assistance	Occupational Medical Service (Bldg 10/6C303, 7 a.m. to 10 p.m., M-F)	496-4411
Emergency Response; Spills, Ambulance	NIH Fire Department (Off Campus)	116 (9-911)

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* Peninsula Laboratories, Inc., (800-922-1516 Toll-free outside California).

USE OF PHENOL IN BIOMEDICAL RESEARCH

(Synonym: Carbolic Acid)

BACKGROUND

Phenol is used in the purification of nucleic acids. It is used to denature proteins in mixtures with buffered aqueous solutions, chloroform, isoamyl alcohol or highly pure, deionized water . The denatured proteins collect at the interphase while most nucleic acids remain in the aqueous phase.

Unfortunately, phenol accounts for many of the chemical injuries reported at the NIH.

CHEMICAL AND PHYSICAL PROPERTIES

Pure phenol is miscible in water and in many organic solvents. It is an acidic compound with a pKa of 9.9 in aqueous solutions. It has low volatility.

Phenol has a distinctive acrid odor with a low odor threshold (0.05 ppm in air). Concentrations below the OSHA determined Permissible Exposure Level of 5 ppm do not result in adverse health effects. A concentration of 8 ppm has been associated with mucous membrane irritation.

TOXIXITY AND HEALTH EFFECTS

Routes of Exposure

In liquid or vapor form, phenol is an irritant to eyes, skin and mucous membranes. It is readily absorbed by the lungs, skin and gastrointestinal tract. Systemic effects may occur from any route of exposure.

Phenol penetrates the skin rapidly. Its absorption is increased with increased temperature and/or concentration. The threshold concentration for human skin damage from phenol is 1.5 percent. The permeability coefficient for a 5 percent phenol solution is 50-fold higher than for a 1 percent solution.

Acute Toxicity

Phenol has a marked effect on tissues. Concentrated solutions of phenol can cause skin necrosis and inflammation. Eye contact may result in severe damage or blindness.

Initially, because of its local anesthetic action, phenol does not cause pain upon skin contact, but produces a whitening of the exposed area. If not removed promptly, the amount of phenol absorbed may produce systemic toxicity.

Early signs of systemic toxicity include muscular weakness and increased blood pressure. These signs, depending on the exposure parameters, may be followed by cardiac dysrhythmia, shortness of breath, and seizures.
Systemic absorption may cause liver and kidney damage, as well as have central nervous system effects. Fatalities have occurred following skin absorption within 10 minutes after 25 percent of an individuals's body surface had been exposed to liquid phenol.

Other Health Effects

Exposures to phenol cause no known genotoxic, reproductive, immunotoxic or carcinogenic effects in humans.

FIRST AID

Skin Exposure

Flush exposed areas immediately with copious amounts of water for at least 15 minutes while removing contaminated clothing, including shoes and socks, to prevent further tissue damage and toxicity. Speed of decontamination is essential in minimizing skin damage and decreasing the risk of systemic toxicity.

Eye Exposure

Immediately flush eyes with water for at least 15 minutes. Hold eyelids open during flushing.

Seek Medical Assistance

Report to the Occupational Medical Service, located in the Clinical Center ACRF (Bldg 10, 6th Floor), as soon as possible. Call the NIH Fire Department (on campus, dial 116) for an ambulance, if needed.

Caution: Do not use alcohol to flush or treat phenol burns. It may exacerbate the injury.

SAFETY PRECAUTIONS

Stock Purchases

Use pre-made/pre-equilibrated liquid phenol to eliminate the risk of exposure during preparation of buffered phenol and other mixtures. Recrystallization of phenol is discouraged. Melting crystals in a water bath increases pressure inside the bottle. When the hot bottle is placed on top of a cold bench, changes in pressure

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and temperature may cause the bottle to break. Order phenol solutions packaged under a dry, inert gas in shatter-proof, plastic coated amber bottles.

Order phenol in the smallest, useful size possible and in the amounts you may need in the next six months or so. This will not only reduce storage space requirements, but also decrease the risk of an accidental exposure, and ultimately lower the cost of chemical waste disposal.

Storage and Handling

Follow temperature and lighting conditions recommended by the manufacturer for storing various mixtures of phenol products. Open bottles in a chemical fume hood in case toxic vapors have accumulated inside. Tighten the cap before returning the bottle to storage.

Transfer phenol from large stock bottles in two stages, first to intermediate size beakers, then to smaller containers. Use containers with a stable base for support. Use a funnel when pouring phenol into a narrow mouth container.

Used glassware should be rinsed thoroughly with water to avoid causing chemical injuries to personnel who autoclave and wash glassware. Activities that may result in the release of phenol vapors should be conducted in areas with adequate exhaust ventilation. Use a chemical fume hood when handling large volumes of phenol.

Personal Protection

Proper protective equipment and clothing should be used to reduce the risk of direct skin or eye contact with phenol. This includes wearing: (1) a fully buttoned lab coat, long pants, and shoes with closed toes and heels; (2) N-Dex disposable nitrile or reusable heavy-duty nitrile gloves (latex and vinyl gloves provide little protection; they should not be used), and (3) chemical splash goggles. Safety glasses provide only limited protection. A face shield should be worn when there is a chance of a chemical splash. These safety items are readily available at the NIH self-service stores.

Safety Shower and Eye Washer

A safety shower and an eye wash fountain should be nearby and easily accessible. Test the eye wash fountain every two weeks to ensure its adequate operation. Only maintenance personnel are authorized to test safety showers.

Chemical Waste Disposal

Collect used or excess liquid phenol and dispose of it as chemical waste in its original or another container (mark **XXX** through the label of the latter). Identify the container's contents, and complete and attach an "NIH Chemical Waste" tag to the container. Chemical Waste Service provides a plastic, non-breakable carboy that may be useful in DNA research labs for decanting of waste phenol. For larger volumes of chemical waste call the Chemical Waste Service to obtain a 5-gallon container.

Collect chemically contaminated solid waste in a clear plastic bag and place it inside a cardboard box. Tie plastic bag after filling, secure box with filament tape, complete an "NIH Chemical Waste" tag (NSN 7530-00-LO7-5985) and attach it to the box. Call the Chemical Waste Contractor to pick up container.

Contact EPB (496-7990) if you have any questions on chemical waste disposal.

REFERENCE

Sullivan, J.B. Jr. and Van Ert, M., Alkylbenzene Solvents and Aromatic Compounds, in *Hazardous Materials Toxicology*, Sullivan, J.B, Jr. and Krieger, G.R. (Eds), Williams & Wilkins, 1992.

For Assistance with	Contact	Telephone
Safety and Health Questions	ICD Occupational Safety and Health Specialist	496-2346
Chemical Waste Questions	Environmental Protection Branch	496-7990
Chemical Waste Pickup	Chemical Waste Contractor	496-4710
Medical Assistance	Occupational Medical Service (Bldg 10/6C303, 7 a.m. to 10 p.m., M-F)	496-4411
Emergency Response; Spills, Ambulance	NIH Fire Department (Off Campus)	116 (9-911)

Mallinckrodt
Material Safety Data

Emergency Phone Number: 314-982-5000

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Mallinckrodt, Inc., Science Products Division, P.O. Box M, Paris, KY 40361

MERCURY

PRODUCT IDENTIFICATION:

Synonyms: Quicksilver; hydrargyrum

Formula CAS No.: 7439-97-6

Molecular Weight: 200.59

Chemical Formula: Hg

Hazardous Ingredients: Mercury

PRECAUTIONARY MEASURES

DANGER! MAY BE FATAL IF INHALED. HARMFUL IF SWALLOWED OR ABSORBED THROUGH SKIN. MERCURY AFFECTS THE KIDNEYS AND CENTRAL NERVOUS SYSTEM.

Do not breathe mist.
Avoid contact with eyes, skin and clothing.
Keep container closed.
Use with adequate ventilation.
Wash thoroughly after handling.

EMERGENCY/FIRST AID

In all cases call a physician immediately. If swallowed, get medical attention immediately. Never give anything by mouth to an unconscious person. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. In case of contact, immediately flush skin or eyes with plenty of water for at least 15 minutes.
SEE SECTION 5.

DOT Hazard Class: ORM-B

SECTION 1 Physical Data

Appearance: Silver-white, heavy, mobile, liquid metal.

Odor: Odorless.

Solubility: Insoluble in water.

Boiling Point: 356.7°C (674°F).

Melting Point: -38.87°C (-38.0°F).

Density: 13.55

Vapor Density (Air = 1): No information found.

Vapor Pressure (mm Hg): 1 @ 126°C (259°F).

Evaporation Rate: No information found.

SECTION 2 Fire and Explosion Information

Fire:
Not considered to be a fire hazard.

Explosion:
Not considered to be an explosion hazard.

Fire Extinguishing Media:
Use any means suitable for extinguishing surrounding fire.

Special Information:
In the event of a fire, wear full protective clothing and NIOSH-approved self-contained breathing apparatus with full facepiece operated in the pressure demand or other positive pressure mode.

SAMPLE

SECTION 3 Reactivity Data

Stability:
Stable under ordinary conditions of use and storage.

Hazardous Decomposition Products:
At high temperatures, vaporizes to form extremely toxic fumes.

Hazardous Polymerization:
This substance does not polymerize.

Incompatibilities:
Acetylenes, ammonia, ethylene oxide, metal oxides, methyl silane, strong oxidants, metal carbonyls.

SECTION 4 Leak/Spill Disposal Information

Do not flush to the sewer. Isolate or enclose the area of the leak or spill. Provide forced ventilation to dissipate fumes. Clean-up personnel should wear respiratory equipment suitable for toxic metal fumes. Transfer to a suitable closed container, preferably glass or plastic, for intermediate storage before reclamation or disposal. Package unreclaimable material for disposal in a RCRA-approved waste facility. Use suction bottle with capillary tube for small amounts, vacuum or other large suction equipment should be provided with mercury vapor absorbant. Cover area of spill with powdered sulfur and floor sweeping compound, before sweeping up. Mercury should be reclaimed insofar as possible.

Reportable Quantity (RQ)(CWA/CERCLA) : 1 lb.

Ensure compliance with local, state and federal regulations.

Mallinckrodt
Material Safety Data

Emergency Phone Number: 314-982-5000

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Mallinckrodt, Inc., Science Products Division, P.O. Box M, Paris, KY 40361.

SECTION 5 Health Hazard Information

A. EXPOSURE / HEALTH EFFECTS

Inhalation:
Highly toxic via this route due to membrane permeability. Transported by blood to brain and other organs. Central nervous system impairment (tremor, irritability, etc.) are symptoms. High vapor concentrations (over 1.2 mg/cu.m.) causes chest pains, dypsnea, and coughing. Mouth and teeth may also be attacked; inflammation of the mouth and loose teeth can result;kidney damage.

Ingestion:
Gastrointenstinal uptake of mercury is less than 5% but its ability to penetrate tissues presents some hazard. Initial symptoms may be thirst, possible abdominal discomfort.

Skin Contact:
Continual contact may allow the absorption of sufficient mercury to produce symptoms as listed in inhalation, above.

Eye Contact:
Not considered a health hazard (OSHA) but mercury droplets can be absorbed through eye tissues.

Chronic Exposure:
Chronic exposure through any route can produce central nervous system damage. May cause muscle tremors, personality and behavior changes, metallic taste, loosening of the teeth, digestive disorders, skin rashes, and kidney damage.

Aggrevation of Pre-existing Conditions:
Persons with nervous disorders, or impaired kidney or respiratory function,or a history of allergies or a known sensitization to mercury may be more susceptible to the effects of the substance.

B. FIRST AID

Inhalation:
Remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Call a physician.

Ingestion:
If swallowed, get medical attention immediately. Never give anything by mouth to an unconscious person.

Skin Exposure:
In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Wash clothing before reuse. Call a physician immediately.

Eye Exposure:
Wash eyes with plenty of water for at least 15 minutes, lifting lower and upper eyelids occasionally. Get medical attention immediately.

C. TOXICITY DATA (RTECS, 1986)

No LD50/LC50 information found relating to normal routes of occupational exposure. Mutation data references cited. Reproductive effects data cited. LDLO Inhalation-rabbit: 29mg/m^3 30H TCLO Inhalation-woman: 150 ug/m^3/46D

SECTION 6 Occupational Control Measures

Airborne Exposure Limits:

For Mercury Vapor:
-OSHA Permissible Exposure Limit (PEL):
0.05 mg/m^3 Ceiling as Hg skin

-ACGIH Threshold Limit Value (TLV):
0.05 mg/m^3 (TWA) as Hg skin

Ventilation System:
A system of local and/or general exhaust is recommended to keep employee exposures below the Airborne Exposure Limits. Local exhaust ventilation is generally preferred because it can control the emissions of the contaminant at its source, preventing dispersion of it into the general work area. Please refer to the ACGIH document, "Industrial Ventilation, A Manual of Recommended Practices", most recent edition, for details.

Personal Respirators: (NIOSH Approved)
If the TLV is exceeded, wear a supplied air, full-facepiece respirator, airlined hood, or self-contained breathing apparatus.

Skin Protection:
Wear impervious protective clothing, including boots, gloves, lab coat, apron or coveralls to prevent skin contact.

Eye Protection:
Use chemical safety goggles and/or a full face shield where splashing is possible. Contact lenses should not be worn when working with this material. Maintain eye wash fountain and quick-drench facilities in work area.

SECTION 7 Storage and Special Information

Keep in a tightly closed container. Store in a cool, dry, ventilated area away from sources of heat or ignition. Protect against physical damage. Wear special protective equipment (Sec. 6) for maintainence break-in or where exposures may exceed established exposure levels. Wash hands, face, forearms and neck when exiting restricted areas. Shower, dispose of outer clothing, change to clean garments at the end of the day. Avoid cross-contamination of street clothes. Wash hands before eating and do not eat, drink, or smoke in workplace.

••

MERCU

SAMPLE

SPOT HAZARD

○ Biological ○ Radiological ● Chemical ○ Physical

PROPER USE OF CHEMICAL FUME HOODS

During the past year, several laboratory accidents have occurred due to the improper use of chemical fume hoods. In two cases, the investigators were prudently working inside properly functioning fume hoods, but inappropriate technique may have disrupted the airflow, contributing to exposures to harmful chemical vapors. Such an event occurred recently when a chemist was using cyanogen bromide in a certified, operating chemical fume hood. Yet, cyanide vapors apparently escaped under the sash, necessitating medical attention for the investigator. In this case, the rear, lower exhaust slot was blocked, creating turbulence that compromised the effectiveness of the fume hood.

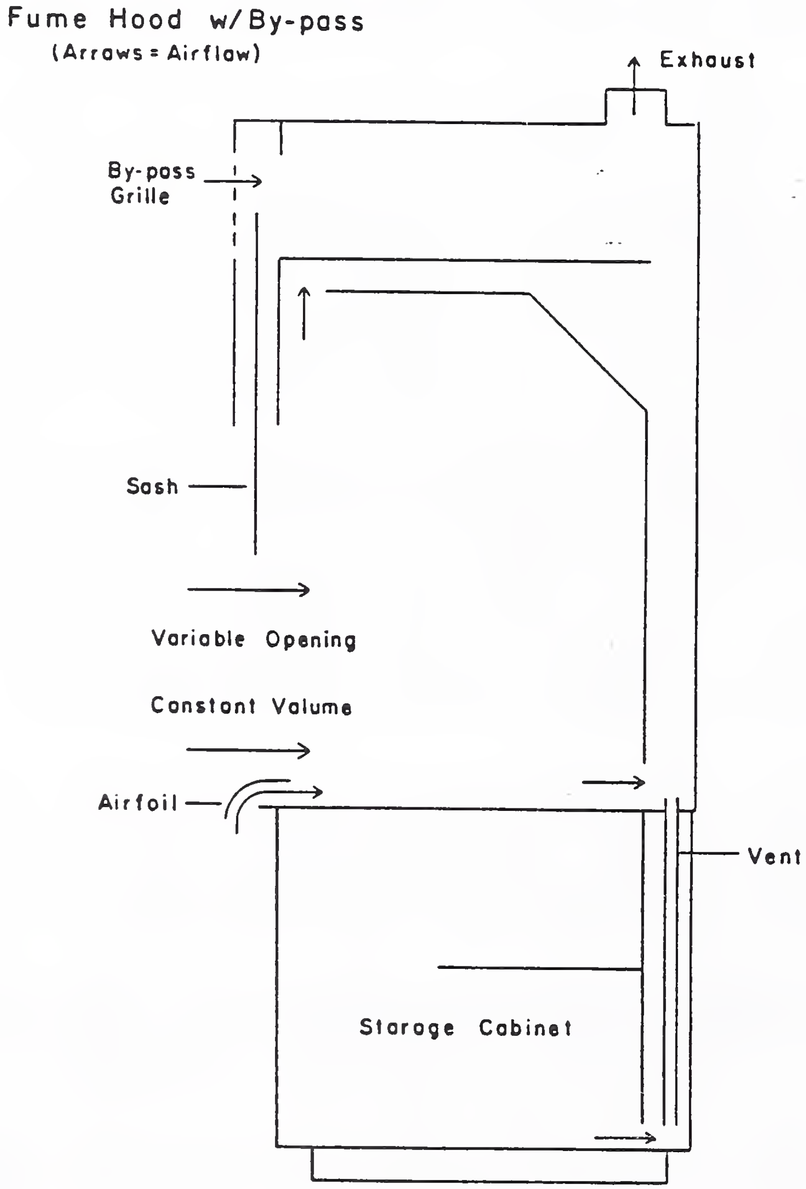
Mishaps such as these can be avoided by observing the following guidelines:

- Chemical fume hoods should be utilized for all operations involving flammable, toxic or malodorous chemical compounds. Conduct all work within the hood at a distance of at least six inches behind the face opening to avoid disruption of airflow from the airfoil (see diagram). Procedures which involve the venting of toxic vapors should be located at the back of the hood and vented in the direction of the air flow.
- The vertical sliding sash should be positioned at the height specified on the hood certification sticker. At this level, the inflow velocity is 100 linear feet per minute which will provide adequate containment for most chemical operations. (If your hood lacks a certification sticker or is marked "Not Suitable..." call the Safety Support Section at 496-3457 for assistance.) The sash will also serve as a physical barrier in the event of chemical splashes or explosions within the hood. For this reason, the sash should be kept below eye level whenever practicable.
- Only the equipment necessary to perform the experiment should be present in the hood. Any apparatus or chemical containers will cause turbulence within the hood which can compromise the containment capability. Under no circumstances should the back exhaust slot be occluded by storage (see diagram).
- Chemicals should not be stored inside the hood. The storage cabinet located under most NIH fume hoods may be utilized for segregated chemical storage, provided that this area is

properly vented into the hood exhaust. If your cabinet is not ventilated, submit a work request to have a vent pipe installed (see diagram). Label all chemicals and other hazardous materials clearly and accurately.

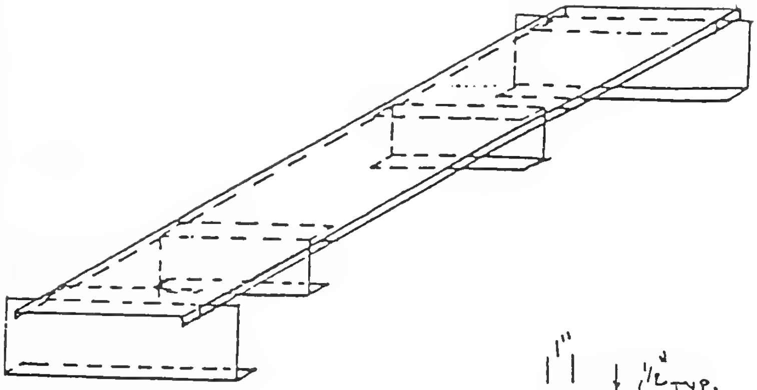
- Minimize the amount of turbulence created by personnel moving in front of the hood, by drafts from open doors and windows, and by heating and air conditioning systems. Such turbulence compromises containment.
- The work surface of the hood should be thoroughly cleaned after completion of the experiment and immediately following any overt spill.

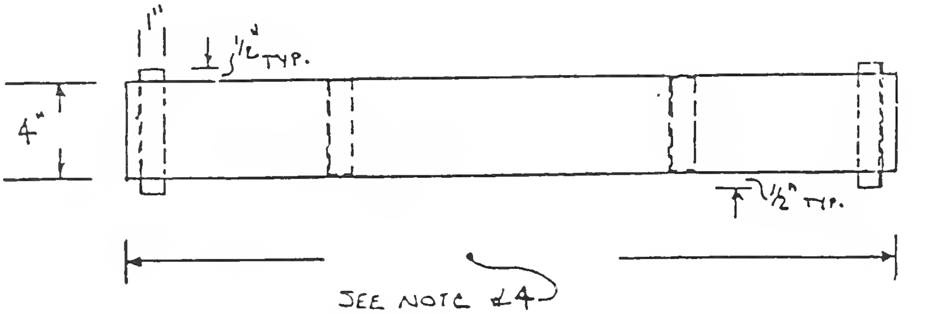
Questions concerning the safe operation of chemical fume hoods should be directed to your BID Safety and Health Consultant on 496-2346.



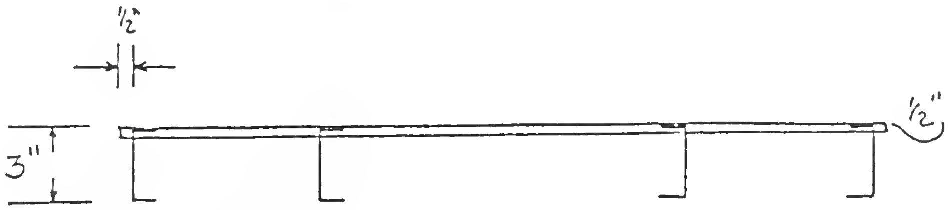
Fume Hood Shelf Details

(attach to work request)





TOP VIEW



FRONT VIEW

Freestanding Chemical Fume Hood Shelf

1. use 20 Ga. S.S. spot welded in place.
2. Hem edges of shelf.
3. Space the two center supports equally.
4. This measurement varies for particular hood, see work request. (inside width =)

NATIONAL INSTITUTES OF HEALTH			
BETHESDA, MARYLAND			
PLANNING & CONTROL BRANCH, DES			
ARCH.	MECH.	ELEC.	PLUM.
REVIEWED AND APPROVED BY REQUESTOR			
DATE			
DR'W'G	SHEET	W.R. NO.	

WASTELINE

Did You Know That: More than 650,000 different chemicals are used and stored by NIH? Unused and outdated chemicals have to be packaged and transported to the NIH chemical recycling center or to a certified disposal site? The cost of the disposal for these chemicals will be close to one million dollars this year?

Chemicals Chemicals Chemicals

Minimization:

There are several ways that you can reduce the cost of disposal. For example, purchase chemicals in the smallest amount needed. Significant disposal fees are then saved.

Ordering Prepared Media and Solutions:

If ingredients are needed to prepare media or solutions to be used for a short period of time or for a small number of experiments, consider ordering the desired item from the Media and Glassware Services Branch. The Media Section, MGSB, will prepare microbiological or tissue culture media and ancillary solutions in any amount in bottles, tubes and plates and in containers provided by the investigator.

Recycling:

Before purchasing a chemical, check with the Environmental Protection Branch's Recycling Service. You may be able to get it without cost. Alternatively, if you have a chemical that has not reached its expiration date or does not have any expiration date and is in an unopened bottle, you can donate it to the Recycling Service.

Don't Dump Chemicals Down the Drain:

There are reasons why this shouldn't be done. Chemicals poured down the drain can damage plumbing systems, upset sewage treatment operations and/or damage the environment. The easiest and safest way to dispose of waste chemicals is to call 496-4710 for pick-up of these items at the work site.

Responsiblity:

Each employee has the responsiblity to dispose of chemicals safely and in accordance with environmental protection requirements.

In a future edition of WASTELINE, we will describe some of the regulations that guide us in the disposal of waste chemicals.

For information contact:

Chemical Disposal
Chemical Waste Contractor
496-4710

Media and Solutions
Media Section, MGSB
496-6017

Recycling Service
Chemical Waste Contractor
496-4710

Division of Safety
Environmental
Protection Branch

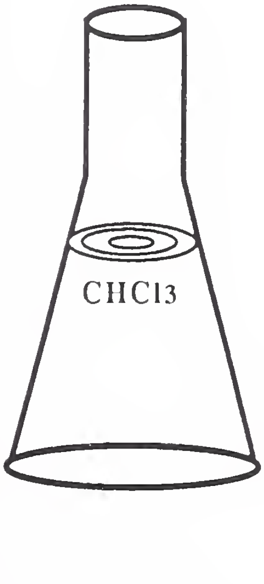
January, 1990

Printed on Recycled Paper

WASTELINE

CHLOROFORM

Trichloroomethano (Italian)
Trichloormethaan (Dutch)
Trichlormethan (Czech)
Chloroformio (Italian)
Chloroforme (French)
Cloroformo (Spanish)
FS9100000 (RTECS)
Methenyl trichloride
Methane trichloride
Methyl trichloride
Formyl tricloride
Methane, trichloro
Trichloromethane
R 20 Refrigerant
Trichloroform
CAS 67663
Chloroform
NCIC02686
Freon 20
CHCL3
R 20
*



No matter what you call it, **CHLOROFORM** is one of the most widely used chemicals in NIH laboratories. It is used in extractions and other biomedical research procedures and also is a decomposition product of some commonly used laboratory chemicals such as trichloroacetic acid (TCA).

Chloroform is a volatile, toxic solvent and a suspected human carcinogen. For waste managers it causes more headaches than any other hazardous substance generated at the NIH.

When radioactive waste contains chloroform it becomes a significant mixed waste problem. Because there are no commercial disposal facilities, this type of mixed waste must be stored indefinitely here on campus

DO NOT POUR solutions that contain ANY concentration of chloroform into drains or sinks. The Washington Suburban Sanitary Commission (WSSC) has established prohibitively low limits for chloroform in the sanitary sewerage system.

The assistance of the entire NIH research community is needed to help solve the chloroform problem. - By following the guidelines on the back, you will do your part to control this toxic compound.

For Information & Assistance:

Mixed and Radioactive Waste Containers and Pickup - *Radioactive Waste Service: 6-4455*

Chemical Waste Containers and Pickup - *Chemical Recycling and Disposal Service: 6-4710*

Radiation Safety and Radioactive Waste *Area Health Physicist, RSB, DS: 6-5774*

Waste Identification, Minimization, Treatment, Recycling and Disposal *EPB, DS: 6-7990*

Laboratory Safety and Inactivation of Infectious Agents *ICD Safety and Health Consultant, OSHB, DS: 6-2346*

Division of Safety
Environmental
Protection Branch

June 1993

GUIDELINES FOR CHLOROFORM CONTAINING WASTE

🛇 DON'T Mix	Do not place chloroform or other nonradioactive chemical wastes in radioactive waste carboys.
! DO Separate	If you must generate radioactive waste that contains chloroform collect it in a container separately from other radioactive wastes. This will minimize the volume of mixed waste that must be stored. [NOTE: Aqueous fractions from extraction procedures involving radioactive materials may contain appreciable levels of chloroform. Collect all fractions from such extractions in a separate container as mixed waste - Don't add them to aqueous radioactive wastes containers.]
! TCA Caution	Trichloroacetic acid (TCA) is widely used in protein precipitation, filter washing and gel fixation procedures. Virtually all wastes containing TCA or its neutralization salts contain significant concentrations of chloroform which form as a decomposition (decarboxylation) product of TCA.
! DO Label	**All radioactive wastes containing chloroform, TCA and its neutralization products or other hazardous chemicals, must be managed as a mixed waste. Attach a Radioactive Waste Pickup Receipt and a NIH Chemical Waste Tag. List all radionuclides and hazardous chemical constituents and their estimated concentrations.**
! DO Substitute	Minimize the generation of radioactive waste containing chloroform and TCA by substituting other less toxic compounds.
🛇 DON'T Pour down the drain.	The direct disposal of chloroform solutions through sinks or drains is prohibited, regardless of the volume or concentration.
! Thank you	**Your cooperation conserves research resources, helps protect the environment, ensures regulatory compliance and reduces utilization of scarce mixed waste storage capacity.**

WasteLine is printed on recycled paper provided by NIH's Printing and Reproduction Branch (PRB). For advice about printing other jobs on recycled paper, call Mr. Samuel Whitmore, 6-6781, of PRB.

WASTELINE

'Double, double, toil and trouble;..."

—Shakespeare, *Macbeth*, Act IV, Scene I.

NIH faces a new breed of waste called "mixed waste". The generation and identification of these concoctions of hazardous chemicals mixed with radioactive material has risen dramatically over the past year. Currently, there are only limited treatment or disposal options for this mixed brew. Mixed waste that cannot be treated or disposed of requires indefinite storage that consumes premium on–campus space which could otherwise be used for other research requirements.

The total cost of managing this waste has risen more than tenfold between 1990 and 1991. These costs will continue to rise throughout the foreseeable future due to limitations of waste treatment technology, the lack of adequate permitted domestic waste treatment facilities, and regulatory restrictions affecting the disposal of these wastes. Unfortunately this situation will increasingly impact the availability of dollars for our important research activities.

'Fire burn and cauldron bubble."

—Shakespeare, *Macbeth*, Act IV, Scene I.

The Mixed Waste Crisis

Background:

While NIH research is directed to the benefit of mankind, there is a "darkside" associated with research activities which is the production of wastes with multiple types of hazardous constituents. These wastes, referred to here as "mixed waste" are also called "multi-hazard waste" at NIH. Mixed waste requires special labeling and handling precautions and includes materials contaminated with radioactive waste and either infectious agents (MPW) or hazardous chemicals or both. In addition to the "double" hazard nature of mixed waste, they are regulated by both the Environmental Protection Agency (EPA) and the Nuclear Regulatory Commission (NRC).

NRC and EPA changes have resulted in two new rules, the Toxicity Characteristic (TC) Rule and Land Disposal Restrictions (LDR) both of which have an impact on NIH.

The TC rule sets limits for toxic substances which if exceeded, must be managed as hazardous waste, as only one example, a waste containing 6.0 mg/l of chloroform is characterized as toxic. The rule requires waste generators to determine levels of these compounds in their waste either by knowledge of the waste generation process or by specific analytical procedures.

The LDR rule prohibits the disposal of untreated wastes such as; solvents and dioxin containing waste as well as, cyanides, polychlorinated biphenyls (PCB), corrosives, halogenated compounds and other restricted hazardous waste. Also, the LDR limits storage of waste, sets mandatory pretreatment standards and prohibits dilution as a means of achieving these standards.

For Information and Assistance:

Mixed and Radioactive Waste Containers and Pickup - *Radioactive Waste Disposal Service* - 490-8100

Chemical Waste Containers and Pickup - *Chemical Recycling and Disposal Service* 496-4710

Radiation Safety and Radioactive Waste *Area Health Physicist, RSB, DS* 496-5774

Waste Identification, Minimization, Treatment, Recycling and Disposal *EPB, DS* 496-7990

Laboratory Safety and Inactivation of Infectious Agents *ICD Safety and Health Consultant, OSHB, DS* 496-2346

Division of Safety
Environmental
Protection Branch

November 1991

The net effect of these developments is that some waste which was formerly managed only as radioactive waste, is now subject to hazardous waste regulations. Chemicals may no longer be disposed of at a radioactive waste disposal site. In addition to these regulatory restrictions, limitations of current waste treatment technology and the lack of adequate permitted domestic waste treatment facilities add to the difficulty and expense of managing mixed waste.

The NIH research community can help by minimizing the generation of mixed waste and by observing separation and identification practices.

Minimization, Segregation and Containment:

Less is better! Regulatory mandates aside, minimization strategies enhance safety, conserve resources, prevent pollution and reduce disposal costs. Important minimization strategies that may be incorporated into the planning and design of laboratory procedures include; volume reduction (use less), avoid over ordering (buy less), and substitution with short lived radionuclides and replacement of hazardous chemicals with nonhazardous ones (generate less).

Segregation and containment are additional minimization strategies that are vital when a laboratory procedure includes the generation of waste. This may be accomplished through the design of a thorough research plan. Strategies may include segregating: • different radioactive wastes from each other (eg. acidic radioactive waste from noncorrosive radioactive waste) • radioactive waste from chemical waste • radioactive waste from infectious agents and other medial pathological waste • radionuclides with short half-lives, ie. less than 65 days, from those having half-lives of 65 days and greater.

Mixed waste should be accumulated in separate containers prior to calling for waste pickup. Appropriate containers for segregating radioactive and chemical wastes are available and delivered free of charge upon request. To ensure effective segregation, correctly identify the containers as "radioactive", "chemical" or "mixed" and place them at safe and convenient locations until your pickup request is serviced.

In general, design your procedures such that radioactive waste is separated from other wastes to the extent practical and avoid mixing radioactive labeled chemically toxic compounds with nontoxic aqueous radioactive waste. By becoming accustomed to using these basic strategies and accurately identifying mixed waste at your work site they will become standard laboratory practice and will allow the NIH to continue to conduct research in a free environment.

Identification:

The NIH has developed two forms to assist researchers in identifying waste materials. Proper mixed waste identification includes the names and estimated concentrations of all hazardous chemicals, the name of the radioactive carrier compound, the name and activity of the radionuclide(s), and the accumulation start date.

This information is used to ensure personnel safety, to determine treatment and disposal methods and is required for regulatory compliance. USE BOTH of the following forms for mixed waste identification:

Radioactive Waste Pickup Receipt - This new label [Form NIH 88-36 (2/91)], introduced in 1991, requests information necessary for processing radioactive wastes. Delivery of the label will be made by the waste contractor at the time of pick-up.

NIH Waste Chemical Tag - This is the familiar yellow tag (Form NIH 2459, NSN 7530-0007-5985) illustrated in the "NIH Waste Calendar". List ALL hazardous compounds, their estimated concentrations, and the date that waste accumulation began in the container.

We need your help to protect the environment and ensure regulatory compliance. Minimize ALL wastes, especially mixed waste, and then segregate, contain, and correctly identify those that must be generated to ensure continued safe, reliable, cost-effective and environmentally sound waste management.

WasteLine is printed on recycled paper provided by NIH's Printing and Reproduction Branch (PRB). For advice about printing other jobs on recycled paper, call Mr. George Mendez, 496-6077, of PRB.

Questions or comments about *WasteLine* may be addressed to the Environmental Protection Branch, Building 13, Room 2W-64 or by calling Mr. Gyula Kovach on 496-7990.

Notes

Canadian Centre for
Occupational Health and Safety

SAFETY INFOGRAM 102

LABORATORIES
BROKEN GLASS

Damaged or broken glassware can cause injuries and spread infections.

Damaged glassware can also lead to serious accidents with hazardous chemicals. As top priority, identify chemical hazards whenever broken glassware causes a spill.

DOs

WEAR eye and face protection when working with glass apparatus.

INSPECT glassware for cracks, stars, or stress lines either visually or with a polariscope before you use it.

REPAIR, fire-polish or discard defective or chipped glassware.

USE extreme care to avoid cuts from broken glass.

REPORT accidents or cuts immediately.

IDENTIFY and isolate the area where glass was broken to prevent further accidents before clean-up.

USE tongs or safety gloves to pick up large pieces of broken glass.

USE a brush and dustpan to pick up small pieces of broken glass.

USE wet cotton held in tongs to sweep up tiny particles of broken glass. A vacuum cleaner can also be used unless the glass was contaminated by a chemical or microbial hazard.

AUTOCLAVE broken glass or disposable glassware exposed to blood or infectious material before disposal. Also autoclave equipment used to pick up broken glass.

DISPOSE of broken glass immediately in special, well-labelled, puncture-proof containers.

DO NOTs

DO NOT USE glassware that is chipped, cracked, or badly etched.

DO NOT PICK UP broken glass with bare hands.

DO NOT LEAVE broken glass among other glassware.

DO NOT WASH broken glassware along with other glassware.

DO NOT MIX glassware or broken glass with regular garbage for disposal.

250 Main Street East Hamilton Ontario Canada L8N 1H6
Phone: (416) 572-2981 Fax: (416) 572-2206

ISSN 0835-818
November 199

Canadian Centre for
Occupational Health and Safety

LABORATORIES

GLASS RODS AND TUBES

Accidents with glass rods and tubes can cause cuts or serious injuries from broken glass.

Follow the proper procedures for working with glass rods and tubes.

DOs

WEAR leather gloves and eye protection when working with glass rods or tubes.

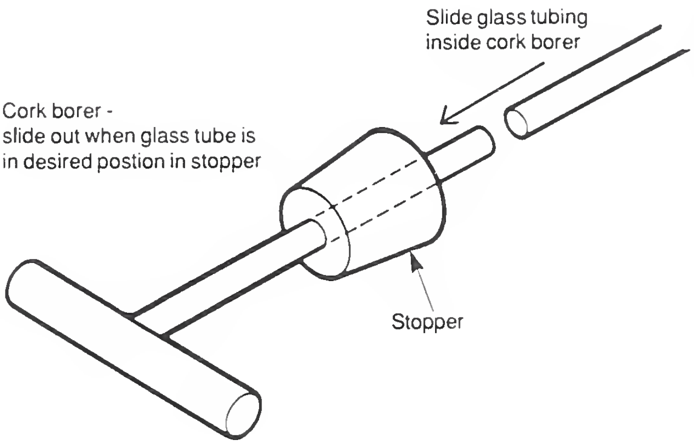
USE the following safe procedures to cut glass rods or tubes:

1. Score the surface with a sharp file to nick one third around.
2. Cover the glass rod or tube with a cloth.
3. Push with thumbs behind the score to snap the rod or tube.
4. Repeat the scoring process if the glass does not break easily. Do not bend it.

FIRE POLISH sharp edges after cutting.

USE the following safe procedures to insert glass rods or tubes into stoppers:

1. Lubricate glass rods or tubes with water or glycerin.
2. Expand the hole inside the stopper by inserting the next largest size cork borer.
3. Place the glass rod or tube inside the borer.
4. Withdraw the borer leaving the rod or tube in place.


Slide glass tubing inside cork borer
Cork borer - slide out when glass tube is in desired postion in stopper
Stopper

USE the following safe procedures to remove glass rods or tubes from rubber stoppers or flexible hoses:

1. Cut rubber stoppers or hoses if the glass rods or tubes become stuck.
2. Discard rubber stoppers or hoses with glass rods or tubes if they cannot be easily separated.

DO NOTs

DO NOT FORCE glass rods or tubes when:

- cutting
- inserting into stoppers or flexible hoses
- removing from stoppers or flexible hose

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Canadian Centre for
Occupational Health and Safety

SAFETY INFOGRAM 103

LABORATORIES

GLASS VACUUM APPARATUS

Glass vacuum apparatus can collapse violently sending broken glass and contents flying. This can happen when the apparatus is hit by an object. It can also occur spontaneously without warning.

Low-pressure systems with water aspirators can be as dangerous as high-pressure systems with vacuum pumps.

Follow the correct procedures for working with vacuum apparatus.

DOs

WEAR goggles or a face shield.

USE only vacuum-approved equipment.

INSPECT large glass vessels for cracks, stars, or stress lines either visually or with a polariscope before use.

CHECK distillation apparatus for leaks by evacuating before applying heat.

ENCLOSE vacuum desiccators in approved shielding containers or wrap properly with friction tape.

USE safety shields with vacuum flasks or distillation apparatus under vacuum.

ALLOW distillation apparatus to cool before releasing the vacuum.

RELEASE vacuum slowly before opening vacuum desiccators.

SLIDE lids off vacuum desiccators. Never lift them off.

USE suitable traps between vacuum sources and vessels under reduced pressure.

SET vacuum vessel within a collection device to hold all contaminated liquid if vessel breaks.

VENT exhausts from oil-filled vacuum pumps to a fume hood.

PROTECT pump and environment with an in-line HEPA filter when working with infectious substances.

DO NOTs

DO NOT EVACUATE regular glassware.

DO NOT MOVE or carry evacuated glass apparatus.

DO NOT USE direct flame to heat distillation apparatus under vacuum.

DO NOT CONTINUE heating vacuum distillation apparatus when vacuum is released. Distillation rate can become excessive when vacuuming is resumed.

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Essential Information on Electrical Safety

Our increased reliance on electrical equipment in the workplace and in our homes has lead to the creation of a new set of potential hazards--many of them unseen.

The improper use or failure of electrical equipment is a leading cause of fires.

By becoming aware of the potential electrical hazards at home and at work, you can significantly reduce the chance of an injury, a fire, or even death.

Important Things To Remember

1) Electricity can be deadly in even relatively small amounts. The "zap" that we associate with an electric current can sometimes be enough to stop a heart beating.

2) Whenever possible, select and use "three prong" grounded or "double insulated" electrical equipment.

3) Inspect the power cord and plug each time the device is used. Place the plug securely into the outlet.

4) Never use power cord or plug which is visibly damaged.
Do not unplug the device by "yanking" the cord out of the wall.

5) Ensure that your hands are not wet or that you are standing in water when use or turn on electrical equipment.

6) A ground fault circuit interrupter (GFI) is recommended for all wet locations-- such as around sinks or in the bathroom.

7) Using the switch, turn off, unplug, or otherwise shut off the power to the device before servicing it, unjamming or cleaning any electrical equipment.

8) Major electrical equipment, circuit breaker panels, light fixtures, or other “hand-wired” equipment must be properly “locked out / tagged out” prior to beginning work.

9) Wherever possible, avoid the use of extension cords; especially the light duty-type made of lamp cord. Multi-plug accepting devices should be avoided as well.

Canadian Centre for
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SAFETY INFOGRAM 107

LABORATORIES

COMPRESSED GAS CYLINDER HANDLING

Before handling cylinders, read all label information and data sheets for the gas.

Handle cylinders with hands and clothing which are free of grit, grease and oil. This reduces slipping and also prevents grit or grease from getting on the nozzle or valve.

Dragging or sliding cylinders can damage them. Roll cylinders on their bottom edge for short distances only.

Locate eyewash stations, safety showers, respirators, and resuscitators nearby but out of the immediate area. It might be contaminated by an accidental release of gas.

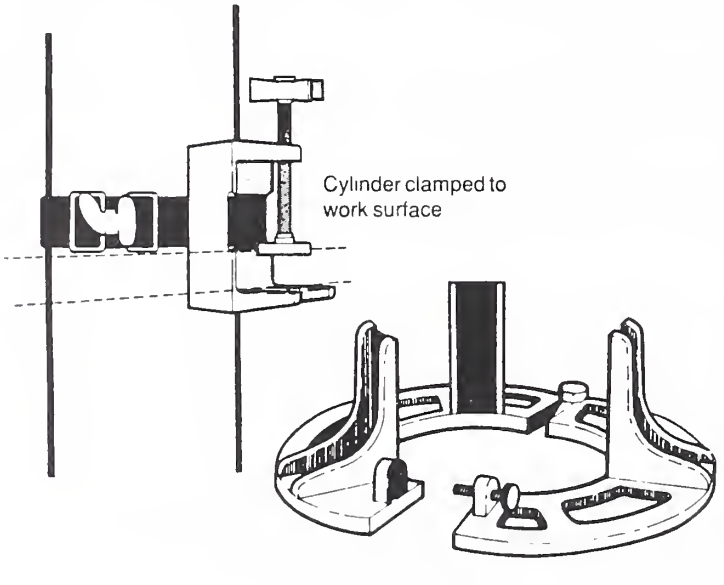
Checklist

DOs

HANDLE all cylinders as if they were full.

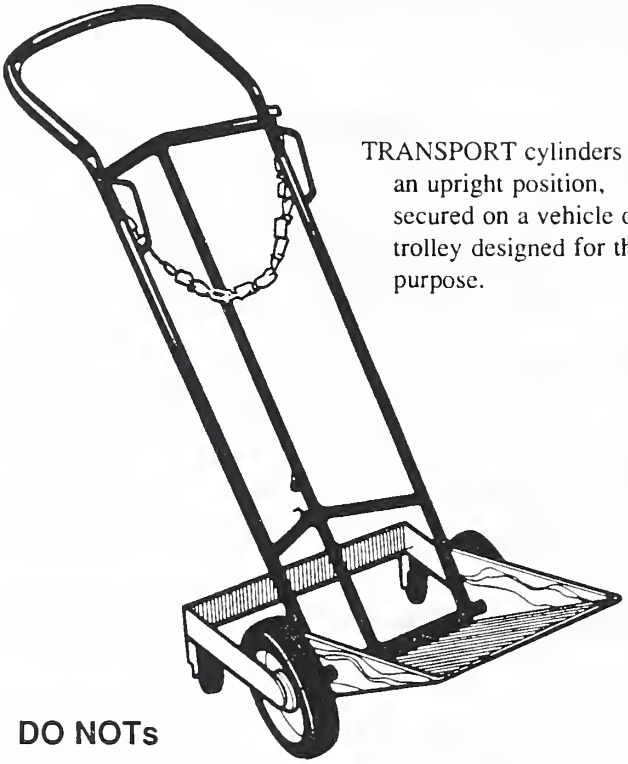
PROTECT cylinders from damage.

SECURE cylinders to a firm support.



TIGHTEN valve protection caps by hand.

MOVE cylinders with valve protection caps on.



TRANSPORT cylinders in an upright position, secured on a vehicle or trolley designed for that purpose.

DO NOTs

DO NOT STRIKE an electric arc on a cylinder.

DO NOT TRANSFER gas from one cylinder to another.

DO NOT LIFT a cylinder by the protection cap.

DO NOT USE cylinders as rollers or supports.

DO NOT DROP cylinders. They could burst, or the valves could break off or become damaged.

DO NOT PLACE an acetylene cylinder on its side.

DO NOT RELY on a cylinder's colour to identify contents. Check the cylinder's stencil and tag.

DO NOT PLACE cylinders where they may become part of an electrical circuit.

Canadian Centre for
Occupational Health and Safety

SAFETY INFOGRAM 108

LABORATORIES

COMPRESSED GAS SET-UP

Regulator Use

Make sure the regulator inlet threads match the cylinder valve outlet threads. Clean cylinder valve before coupling with regulator. Connect the regulator to the cylinder's outlet valve.

Fit flashback arrestors or check valves between cylinders and equipment when using a flammable gas or when doing a procedure at cylinder pressure.

Use regulators designed for the contents of the cylinders. Regulators are not to be used to stop the flow of gas from the cylinder. Close the main cylinder valve when the gas is not being used. Regulators can leak and allow excessive pressures to develop in equipment.

Set-up Consideration

Work in well-ventilated areas to reduce the hazards of toxic, flammable and corrosive gases.

Use a gas detector to warn of leaks when handling toxic gas cylinders.

When using corrosive gases, work the cylinder valve frequently to prevent freezing. Flush regulators and valves with dry air or nitrogen after each use in corrosive service. Do not leave control devices on these cylinders, except when cylinders are in frequent use. Before discharging corrosive gases into a liquid trap, always use a check valve or a vacuum break device to prevent a dangerous suck-back.

Never connect a gas cylinder directly to a reaction vessel. Always properly connect a surge vessel and a system of traps.

Remove regulators from empty cylinders. Replace the protective caps. Mark the cylinder "MT." Never empty a cylinder completely. Leave a slight pressure to keep contaminants out.

Checklist

DOs

WEAR safety glasses to prevent eye injury from equipment failure.

STAND to one side and away from regulator gauge faces when opening cylinder valves.

LEAVE key wrenches on cylinders in use so the cylinders can be closed quickly.

ENSURE that connections between the regulators and cylinder valves are tight.

CHECK the accuracy of regulator pressure gauges at least yearly.

BOND and ground all cylinder lines and equipment used with flammable gases.

DO NOTs

DO NOT USE pipe wrench or pliers for attaching regulator to cylinder. Use wrench of proper size.

DO NOT USE a regulator intended for use with another gas.

DO NOT USE oil or grease to lubricate tight threads. Oil or grease on regulators or fittings can cause an explosion.

DO NOT USE cylinders without a regulator. Cylinders are at pressures greater than most laboratory equipment can withstand.

DO NOT USE compressed gas to blow away dust or dirt.

DO NOT EXTINGUISH a combustible gas flame until the source of gas has been shut off. The gas can re-ignite and explode.

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Canadian Centre for
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SAFETY INFOGRAM 109

LABORATORIES

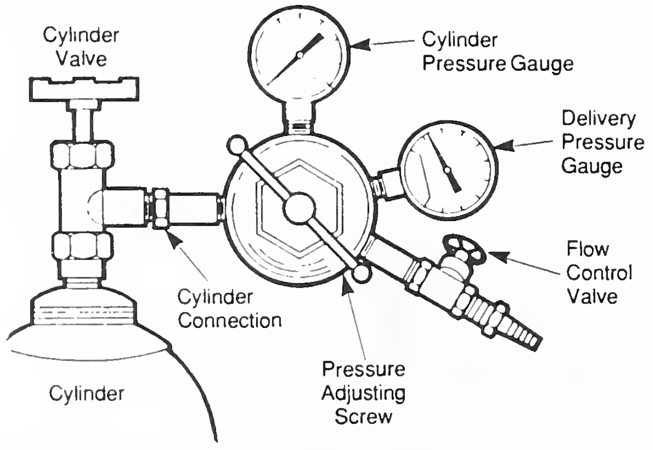
COMPRESSED GAS REGULATOR USE

ON

1. Check that flow control valve is closed.
2. Turn the pressure adjusting screw counterclockwise until it turns freely.
3. Slowly open the cylinder valve fully.
4. Check the cylinder pressure gauge for expected value. A difference indicates that valve, safety device, or plugs are leaking.
5. Turn the pressure adjusting screw clockwise until the required pressure is reached.
6. Control the gas flow by the flow control valve.

OFF

1. Shut cylinder valve first.
2. Allow residual gases in valves to drain.
3. Shut pressure adjusting screw when gauges fall to zero .
4. Close the flow control valve.



DO NOT ADJUST pressure by using the regulator to control flow rates. This defeats the purpose of the pressure regulator. When higher flows are obtained by this way, the pressure may be above the design of the system.

Maintenance Check

CHECK non-corrosive gas regulators (nitrogen and helium) monthly.

CHECK corrosive gas regulators (chlorine and hydrogen sulfide) weekly.

The procedure for checking regulators is as follows:

DRAIN all pressure from the system. Gauges should read zero.

OPEN cylinder valve and turn pressure adjusting screw counterclockwise until it turns freely. The high pressure gauge should register the cylinder pressure. The delivery outlet pressure gauge should not indicate any pressure.

CHECK the delivery pressure gauge to ensure it does not indicate a pressure increase after waiting ten minutes with the regulator outlet valve closed.

TURN the pressure adjusting screw clockwise until delivery pressure is indicated. Failure to attain a proper delivery pressure setting or abnormal adjustment of the screw indicates improper operation.

CLOSE the cylinder valve and observe the pressure on both cylinder contents and delivery sides of the regulator. A drop in the pressure reading after ten minutes indicates a leak in the system.

ENSURE that only the supplier makes the repairs.

Refer to SAFETY INFOGRAM I08, Compressed Gas Set-Up, for further information on regulators.

Canadian Centre for
Occupational Health and Safety

LABORATORIES

CRYOGENIC FLUIDS

Cryogenic fluids, such as liquid argon, liquid nitrogen or liquid oxygen, are used to obtain extremely cold temperatures. Very small amounts of liquids produce large volumes of gas.

Fire and explosion may result from certain escaping cryogens such as oxygen and hydrogen. Even materials normally non-combustible will ignite if in oxygen-rich atmosphere.

Cryogens cause cold burns and frostbite. Eyes are damaged by even brief exposures. Rapid evaporation can lower available oxygen in the immediate area, creating oxygen-deficient atmospheres.

Thermally insulate vessels containing cryogens from sources of heat. When not in use, secure containers used for transporting cryogenic fluids to a support.

Personal Protection

WEAR insulated gloves when handling anything that may have been in contact with a cryogenic fluid. Fit gloves loosely so that they can be thrown off quickly if liquid spills or splashes into them.

WEAR a non-porous, knee-length laboratory coat or full-length apron which fastens at the back. Make sure coats and aprons do not have pockets or cuffs.

WEAR boots with tops high enough to be covered by a cuffless pant leg.

WEAR full face protection including safety glasses and goggles.

Checklist

DOs

CONTROL access and post warning signs where cryogens are stored or used. Make sure walls and floors of storage areas are made of concrete.

ENSURE proper ventilation in areas where cryogens are stored or used, to reduce the risk of explosion, fire or asphyxiation.

VENT vessels containing cryogens with an approved safety device which permits excess gas to escape.

FILL containers with only the liquids they were designed for and in accordance with the manufacturer's instructions. Label contents of each container.

USE a filling device, a face shield, and insulated gloves when pouring a liquid cryogen.

STAND clear of boiling or splashing cryogens.

PERFORM operations slowly to minimize boiling and splashing when charging a warm condenser or when inserting objects into a cryogenic liquid.

DO NOTs

DO NOT STORE oxygen with flammable gases.

DO NOT WELD or heat containers if they contain a cryogen.

DO NOT WEAR watches, rings, bracelets, or other jewellery.

DO NOT FILL containers higher than the indicated level.

DO NOT ROLL containers. Move only with proper trolleys or carts.

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National Institutes of Health

Division of Safety
1993

WASTE DISPOSAL

Accidents, Spills, Fire: Dial 116
OFF CAMPUS: DIAL 9-911

General Waste		Assistance 6-7990
Chemical Waste	Pick-up 6-4710	Assistance 6-7990
Multi-Hazard / Mixed Waste		Assistance 6-7990
Medical Pathological Waste (MPW) ☣		Assistance 6-2346
Radioactive Waste ☢	Pick-up 6-4451	Assistance 6-5774

Waste Collection & Disposal Supplies

Available via DEL-PRO or NIH Store:

Item	Stock Number	Size	Used for:
Autoclave bag, biohazard w/process indicator	**NSN-6530-01-282-6378**	small	Autoclave MPW/media/labware
Autoclave bag, biohazard w/process indicator	**NSN-6530-01-218-4644**	large	Autoclave MPW/media/labware
Autoclave bag, biohazard w/process indicator	**NSN-6530-01-142-2255**	medium	Autoclave MPW/media/labware
Bag closures, plastic bag ties	**NSN-8105-00-L04-2610**	12"	Seal bags w/animal carcass/bedding
Bag, black plastic/polyethylene	**NSN-8105-01-044-5727**	19.5"X44.5	Animal carcasses/tissue/bedding
Bag, clear plastic	**NSN-8105-01-195-8730**	13" X 24"	Collect chemically contaminated solids
Biohazard label	**NSN-7530-00-L07-2375**	1" X 3"	Warn of biohazard material
Caution label Radioactive Material tape	**NSN-7690-00-833-0318**	roll	Identify radioactive material
Chemical Waste Tag	**NSN-7530-00-L07-5985**	package	Identify chemical waste
Disposable labware & broken glass box	**NSN-8115-01-154-2305**	floor	Collect disposable labware/broken glass
Disposable labware & broken glass box	**NSN-8115-01-122-1772**	bench	Collect disposable labware/broken glass
Filament tape	**NSN-7510-00-290-8036**	roll	Close waste bags/seal boxes
MPW Box kit	**NSN-8115-00-L04-0680**	kit	MPW collection and disposal
Pads, absorbent paper	**NSN-8135-01-025-2532**	18" X 20"	Absorb residual radioactive liquid
Plastic Bag, clear	**NSN-8105-00-826-6468**	30" X 40"	Dispose of chemically contaminated solids
Radioactive Waste Pickup Receipt	**NSN-7530-00-L07-8835**	each	Identify radioactive waste
Sharps container, puncture resistant	**NSN-6530-01-294-2865**	small	Collect sharps for disposal
Sharps container, puncture resistant	**NSN-6530-01-274-5099**	medium	Collect sharps for disposal
Sharps container, puncture resistant	**NSN-6530-01-196-0283**	large	Collect sharps for disposal
Sodium hypochlorite (bleach)	**NSN-6810-00-598-7316**	1 gal	Disinfect/inactivate pathogen(s)
Wescodyne povidine-iodine based solution	**NSN-6840-00-526-1129**	bottle	Disinfect/inactivate pathogen(s)

Available from Radioactive Waste Service (6-4451):

Carboy plastic container	To collect aqueous radioactive waste
Step-can	To collect solid radioactive waste

Available from Chemical Disposal Service (6-4710):

Container	To collect aqueous hazardous/mixed waste and bulk chemicals > 4 L
Safety can	To collect flammable chemical waste

General Waste

Definition: To be considered "general waste," waste material must be free of any **actual** or **apparent** contamination: pathological/infectious, radioactive or hazardous chemical. In some cases, after disinfection or decontamination, previously contaminated material may be treated as general waste.

Examples of general waste include:
- Office waste—paper, boxes, folders
- Cloth, paper, plastic or glass items (uncontaminated or decontaminated)
- Decontaminated media or labware
- Animal bedding (uncontaminated)

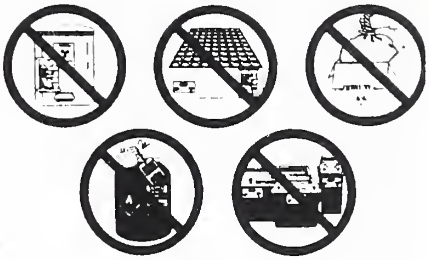
Packaging Guidelines

General Instructions:

***Never* dispose of as general waste:**

- Items which are labeled with either a: biohazard *or* radioactive label
- Sharps (needles, syringes, scalpel blades, etc.)
- Material which is considered MPW (see MPW Section for definition)
- An MPW box regardless of content

***Never* use the MPW box to dispose of general waste**



If Discarding:	Do This:	Collection:
Office Waste	• Place in plastic-lined wastebasket • For larger items, label as "*trash,*" inform Housekeeping	Housekeeping will collect
General Office Paper Recycling	White Paper Recycling Recovery areas: Bldgs. 1, 13, 31 • Put only white paper into Paper Recycle Containers	Recycle containers are collected on a scheduled basis
Building 10	• Collect paper in cardboard box • When full, close, seal and label "*Recycle Paper*"	CC Housekeeping will collect
Other Campus Buildings	• Collect paper in cardboard box • When full, close, seal and label "*Recycle Paper*"	Call Transportation Branch (6-1546) for pick-up
Uncontaminated Glass/Plastic Labware	• Place in the Disposable Labware and Broken Glass Box; Bench size: **NSN-8115-01-122-1772** or Floor size: **NSN-8115-01-154-2305** • Close container and secure with filament tape **(NSN-7510-00-290-8036)**	Housekeeping will collect Disposable Labware and Broken Glass Box
Animal Bedding—Uncontaminated	• Fill polyethylene bag **(NSN-8105-01-044-5727)** 3/4 full or up to 40 pounds • To close bag, twist top and seal securely with either: Plastic bag closure tie **(NSN-8105-00-L04-2610)** or Filament tape	Animal care personnel collect
Media		
Liquid Media—Not Contaminated	• Pour liquid into sink • Put empty container, if not reusable, in Disposable Labware and Broken Glass Box	Housekeeping will collect Disposable Labware and Broken Glass Box
Liquid Media—Biologically Contaminated	• Inactivate biologic agents by decontamination with either: steam autoclave, chemical disinfectant (see MPW Section for procedures) • Pour liquid into sink • Put empty container, if not reusable, in Disposable Labware and Broken Glass Box	Housekeeping will collect Disposable Labware and Broken Glass Box
Solid Media	• Place in autoclavable *"Biohazard"* bags imprinted with process indicator: small: **NSN-6530-01-282-6378,** medium: **NSN-6530-01-142-2255,** large: **NSN-6530-01-218-4644** • Autoclave and cool (see MPW Section for procedure) • Put bag in Disposable Labware and Broken Glass Box	Housekeeping will collect Disposable Labware and Broken Glass Box

Chemical Waste

Definition: Nonradioactive chemicals and solid wastes contaminated with hazardous chemicals

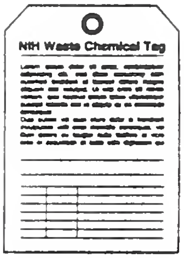
Examples
- Waste and opened surplus chemicals
- Antineoplastic agents and other prescription drugs (noncontrolled substances)
- Empty drums and other containers with a capacity of 5 gallons and greater
- Broken thermometers and other items containing mercury
- Nonreturnable gas cylinders and lecture bottles (18 inch length maximum)
- Residue of spill clean-up materials
- Nonradioactive lead shielding and lead scrap
- Photographic film processing solutions
- Used oil of all types
- Pesticides
- Spent solvents
- Batteries

ackaging Guidelines

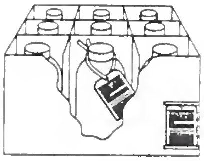
eneral Instructions:

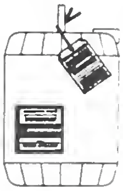
aste chemicals should not be
iscarded:
- *into sink drains*
- *as general waste*
- *as MPW*

- When completing the **NIH Chemical Waste Tag**, identify all major constituents and hazardous components by chemical name, not by acronym or brand name
- Instructions below specify how different containers are to be marked or tagged
- Keep aqueous waste separate from organic solvents
- Retain chemicals in laboratory while awaiting pick-up
- **NIH Chemical Waste Tags** may be obtained from Self Service Stores/DELPRO **NSN-7530-00-L07-5985**



Discarding:	Then:
hemicals in: *Original* Container	On container print: *"Waste," discard date* If original label is loose or illegible, complete and attach an **NIH Chemical Waste Tag**
Container *Other* than Original	Mark "XXX" through label Complete and attach an **NIH Chemical Waste Tag**
Multiple Containers	Multiple containers of compatible chemicals may be placed in a single carton for disposal Ensure containers are sealed Leave tops of containers visible and lid of box unsecured Complete Room/Building information of an **NIH Chemical Waste Tag** Attach tag to the side of the box Call for pick-up (6-4710) no later than 60 days after disposal date on first container placed in box
lk Chemicals	Call the NIH Chemical Disposal Service (6-4710) to obtain containers for volumes greater than 4 liters Add only compatible chemicals to a single container Complete and attach to the container an **NIH Chemical Waste Tag** Record on the **NIH Chemical Waste Tag** each addition of chemical to the container Call for disposal no later than 60 days after the first addition of chemical waste to the container
mmable Solvent and Aqueous xtures with Flammable Solvents *o not place heavy metal compounds, idizers, phenols, strong acids or bases the safety can*	Call the NIH Chemical Disposal Service (6-4710) to obtain a solvent safety can Complete and attach to the container an **NIH Chemical Waste Tag** Record on the **NIH Chemical Waste Tag** each addition of chemical to the container Fill safety can to the "fill" line Store away from ignition and heat sources Call for disposal no later than 60 days after the first addition of chemical waste to the container





Chemically Contaminated Solid Waste

Do not use an MPW box or orange autoclave bag

- Place materials in a clear plastic bag:
  medium: **NSN-8105-01-195-8730**,
  large. **NSN-8105-00-826-6468**
- Close bag with either filament tape or bag closure tie
- Place bag in a plain cardboard box
- Secure box with filament tape
- Complete and attach an **NIH Chemical Waste Tag**

Disposal Information

Retain chemicals in laboratory awaiting pick-up. Do not place waste containers in corridors.

For Assistance About:	Call:	At:
Chemical Spills On Campus	NIH Fire Department	116
Off Campus	Montgomery County Emergency Services and then the NIH Fire Department	9-911 6-2372
Disposal of Narcotics and Other Controlled Substances	Crime Prevention Branch, Division of Security Operations	6-9818
Explosive or Reactive Chemicals	Environmental Protection Branch	6-7990
Ordering Chemical Waste Containers	Chemical Disposal Service	6-4710
For Disposal and Pick-up Chemical Waste	Chemical Disposal Service	6-4710

Multi-Hazard/ Mixed Waste

Definition: Waste with multiple types of hazardous constituents including contamination with radioactive waste and either infectious agents, (MPW) or hazardous chemicals, or both.

Examples:
- Aqueous radioactive wastes with trace levels of chloroform or toxic heavy metals
- Radioactive methanol/acetic acid solutions from HPLC or gel rinse procedures
- Spent cocktail from continuous liquid scintillation counting
- Radioactive trichloracetic acid solutions from protein precipitations
- Phenol/chloroform mixtures used to extract DNA from radiolabled cells
- Vacuum pump oil contaminated with radioactive materials
- Spent chromic acid from cleaning of glassware contaminated with radioactive materials
- Chemical or radioactive wastes containing blood products
- Contaminated lead

Packaging Guidelines

General Instructions:

- Avoid generating mixed waste or combining chemical, radioactive and infectious wastes.
- If generation of mixed wastes cannot be avoided:
  - Keep volume to minimum
  - Keep liquid and solid wastes separate
  - Keep aqueous and organic solvents separate
  - Adjust the pH of waste solutions to between 6-10
  - Keep short half-life (<100 days) isotopes separate from longer half-life isotopes
- Identify all hazardous constituents—see Chemical Waste, MPW and Radioactive Waste sections for required labels/tags
- Do not place waste containers in corridors Retain waste containers in laboratory awaiting pick-up.

Do Not Combine:

- *chloroform or trichloroacetic acid (TCA) with any other liquid waste*
- *reactive chemicals such as strong oxidizers with organic compounds*

If Discarding:	Then:	Disposal and Additional Information
Waste Containing Infectious agent *and* Radioactive material	• Inactivate infectious agent • Seek advice before implementing inactivation procedure • Dispose of waste according to instructions for Radioactive Waste	 • Call ICD Safety & Health Consultant, 6-2346 • Radioactive Waste Service, 6-4451
Infectious agent *and* Hazardous chemical(s)	• Inactivate infectious agent • Seek advice before implementing inactivation procedure • Dispose of waste according to instructions for Chemical Waste	 • Call ICD Safety & Health Consultant, 6-2346 • Chemical Disposal Service, 6-4710
Hazardous chemical(s) *and* Radioactive material	• Use Aqueous Mixed Waste container from Chemical Disposal Service • Affix **NIH Chemical Waste** tag and **Radioactive Waste Pick-up Receipt** • Dispose of waste according to instructions for Radioactive Waste	• Chemical Disposal Service, 6-4710 • Radioactive Waste Service, 6-4451
Infectious agent *and* Radioactive material *and* Hazardous chemical(s)	• Inactivate infectious agent • Seek advice before implementing inactivation procedure • Affix **NIH Chemical Waste** tag and **Radioactive Waste Pick-up Receipt** • Dispose of waste according to instructions for Radioactive Waste	 • Call ICD Safety & Health Consultant, 6-2346 • Radioactive Waste Service, 6-4451
Radioactively contaminated lead	• Decontaminate the lead • Remove or deface all **Caution Radioactive Material** labels	 • Radioactive Waste Service, 6-4451

Disposal Information

For Assistance About:	**Call:**	**At:**
Procedures for Inactivation of infectious agents	ICD Safety and Health Consultant	6-2346
Decontamination—radioactivity	Area Health Physicist	6-5774
How to Minimize Mixed Waste:		
Radioactive Waste	Area Health Physicist	6-5774
Chemical Waste	Environmental Protection Branch	6-3537
Infectious Agents	ICD Safety and Health Consultant	6-2346
Mixed Waste Spill:		
On Campus	NIH Fire Department	116
Off Campus	Montgomery County Emergency Services,	9-911
	then: • NIH Fire Department	6-2372
	• Radiation Safety Branch (If radioactive material involved)	6-5774
Explosive or Reactive Chemicals	Environmental Protection Branch	6-7990
Ordering Waste Containers to Collect:		
	Radioactive Waste Service:	6-4451
Aqueous Radioactive Waste	• Carboy plastic container	
Solid Radioactive Waste	• Step-can	
	Chemical Disposal Service:	6-4710
Flammable Chemical Waste	• Safety Can	
Aqueous Hazardous/Mixed Waste	• Container	
Bulk Chemicals >4L	• Container	
Pick-up at Your Workplace for:		
Radioactive Waste	Radioactive Waste Service Information needed to request pick-up: • Authorized user name and RSB ID number • Location—Building and room • Type of waste—liquid, solid, LS vials, etc. • Radionuclide(s) and estimated amount of activity for each • Chemical waste constituents • Special instructions	6-4451
Chemical Waste	Chemical Disposal Service	6-4710

Medical Pathological Waste (MPW)

Definition: Waste that, because of the **actual** or **perceived** presence of pathogenic agents, requires containment and/or treatment to prevent occupational or environmental exposure.

This includes waste such as animal carcasses and tissues, needles, syringes, scalpels, razor blades or other material that looks like medical waste but which may not actually contain or be contaminated with pathogenic agents. The animal carcasses and tissues may be seen as or may become putrescent. It is difficult if not impossible to determine if sharps of any type are biologically contaminated. Sharps also present a physical hazard. These items contribute to the aesthetic degradation of the environment and must be disposed of as MPW.

Examples:
- Microbiological cultures
- Pathological waste (organs and tissue from humans or animals)
- Clinical specimens (urine, feces, blood)
- Animal bedding contaminated
- Disposable clothing, towels, sorbent material, and similar materials potentially contaminated with pathogenic agents or trace amounts of cytotoxic agents including empty vials and drug dispensing apparatus that contained cytotoxic drugs
- Anatomical waste
- Animal carcasses and tissues
- Waste from surgical/autopsy suites
- Tissue cultures
- Sharps (scalpels, razor blades, pasteur pipettes) and all needles and syringes

Packaging Guidelines

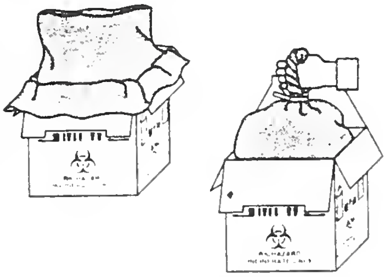
General Instructions:

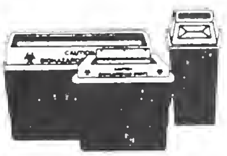
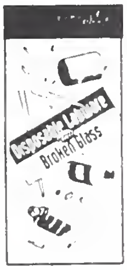
For MPW which contains or is contaminated with radioactive material, for disposal procedures refer to the Multi-Hazard/Mixed Waste Section.

- ***All* MPW must be double bagged**
- Avoid contaminating the outside of the MPW box to protect personnel handling the boxes
- General waste such as soda cans, papers, cardboard, bottles, etc. should never be placed in the MPW box
- The MPW box should never be used for storage or movement of laboratory equipment, office equipment, or household items
- The MPW box comes as part of a kit that contains five boxes, ten bags and plastic bag closure ties. The MPW box kit makes it easy to comply with the requirement to double bag all MPW waste. The MPW box kits,are available through the NIH Self-Service Store in Building 35 and NIH Stock Catalog **NSN-8115-00-L04-0680**

MPW Burn Box Packing Procedures

1. Fold the flaps down on the outside of the box
2. Place *two* plastic bags (one inside the other) into the MPW Box and pull the bag tops down over the flaps
3. Limit loading of each box to 40 pounds of MPW or 3/4 full
4. Seal each bag *separately*. Twist plastic bag at the top; bend the twisted portion to form a loop and seal using the plastic bag closure tie
5. Close the box. Fold Flap A down into box, fold the B Flaps over Flap A, push Flap C down to lock with Flap A
6. *Print* building and room number on box top label area



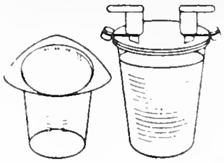
If Discarding:	Then:
"Sharps" (needles, syringes, scalpel blades, etc.) *Do not recap, bend, remove or clip needles*	• Place "Sharps" in one of these puncture resistant containers small: **NSN-6530-01-196-0284,** medium: **NSN-6530-00-L03-5923,** large: **NSN-6530-01-196-0283** • Fill 3/4 full and snap lid closed • Dispose of container in an MPW box 
Labware Disposal (metal, plastic, glass)	• Sodium hypochlorite **(NSN-6810-00-598-7316)** or Wescodyne **(NSN-6840-00-526-1129)** are suitable chemical disinfectants • Submerge the labware for 30 minutes in disinfectant • Decant disinfectant • Dispose of labware in a Disposable Labware & Broken Glass Box Bench size: **NSN-8115-01-122-1772** or Floor size: **NSN-8115-01-154-2305** • Dispose of the box as general waste • **Glassware/labware that cannot readily be decontaminated must be disposed of as MPW** 

Liquids

Cell Culture Media

- Cell culture media must be decontaminated before disposal. This may be accomplished by adding disinfectant directly to vessel or treating pooled spent media. Decontaminated media may be disposed of via a sink
- For each 100 ml of media, use either:
  10 ml of sodium hypochlorite *or* Wescodyne according to manufacturer's directions
- Dispose of cell culture vessel as Disposable Labware

Blood and Body Fluids



- Blood and body fluids should be decontaminated before disposal. Decontamination can be accomplished by using 10ml of sodium hypochlorite per 100 ml of blood or body fluid
- Decontaminated blood and body fluids may be disposed of via a sink

Steam Sterilization

General Instructions:

MPW, except for dense materials (e.g., body parts, animal carcasses or tightly packed material) can be autoclaved and then disposed of as general waste

Procedure for MPW contained in a biohazard autoclave bag with process indicator

1. Use autoclavable *"Biohazard"* bags imprinted with process indicator:
   small: **NSN-6530-01-282-6378,**
   medium: **NSN-6530-01-142-2255,**
   large: **NSN-6530-01-218-4644**
2. **Add 250 ml water to Biohazard Bag *before* closing**
3. Place in pan for transporting and autoclaving
4. Process for 30 minutes at 121°C (15 psi)
5. Cool and discard bag and contents as general waste

Collection Services

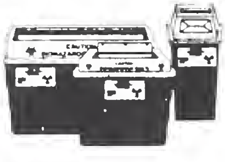
If Located In:	How:	When:
Building 10	Packaged and labeled MPW will be picked up by CC Housekeeping from laboratories and patient care units	7:30 a.m. - 4:00 p.m. Monday through Friday (except holidays)
Building 10C ACRF Animal Facilities	Packaged and labeled MPW will be picked up by animal caretakers from the 10C elevator lobby and transported to the B2 cagewash area	7:30 a.m. - 4:00 p.m. Monday through Friday (except holidays)
NIH Buildings On Campus Including Animal Facilities	Take packaged and labeled MPW to building loading dock or hold in designated cold room for next pick-up	7:30 a.m. - 2:30 p.m. Monday through Friday (except holidays)
NIH Buildings Off Campus	Take packaged and labeled MPW to building loading dock or hold in designated cold room for next pick-up	Scheduled days

Note After 2:30 p.m., Monday through Friday, weekends or holidays, animal carcasses and putrescent material should be stored in an appropriate refrigerator or freezer until disposal is available. Use explosion-safe units if the animals have been euthanized with ether.

For Assistance About:	Call:	At:
Decontamination Procedures	ICD Safety and Health Consultant	6-2346
Packaging MPW	ICD Safety and Health Consultant	6-2346
Pick-up Building 10	Clinical Center Housekeeping Department	6-2417
Pick-up All Buildings Except Building 10	Environmental Protection Branch	6-7990

"Sharps"
needles/syringes/scalpel blades/
razor blades, etc

- Place all "sharps" in one of these puncture resistant containers
  small (**NSN-6530-01-294-2865**),
  medium (**NSN-6530-01-274-5099**),
  large (**NSN-6530-01-196-0283**)
- Attach a **Caution—Radioactive Material** label
- Attach a **Radioactive Waste Pickup Receipt**
- Containers with radioactive "sharps" should *not* be disposed of in a MPW box
- Call Radioactive Waste Service for pick-up (6-4451)

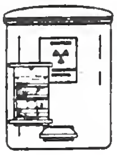


Lead

- Call Radioactive Waste Service for assistance (6-4451)

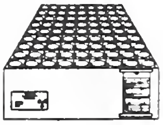
Other Types of Dry/Solid Material

- Use labelled step-can containers available from Radioactive Waste Service
- Attach a **Radioactive Waste Pick-up Receipt**



Liquid Scintillation Vials

- Tightly secure caps of all vials
- Segregate vials according to radionuclide. Vials containing H-3 and/or C-14 may be disposed of together
- Place capped vials in original shipping tray or box
- Attach a **Caution—Radioactive Material** label
- Attach a **Radioactive Waste Pick-up Receipt**

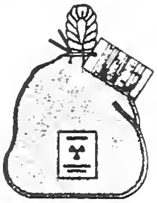


Animal Carcasses and/or Tissues

Refrigerate or freeze if held longer than 4 hours; freeze if held more than 24 hours

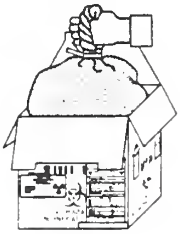
Small Animals and/or Tissues

- Double bag with plastic bags (**NSN-8105-01-044-5727**)
- Close each bag separately. Twist top of bag; bend twisted portion to form a loop and close with a plastic bag closure tie (**NSN-8105-00-L04-2610**)
- Attach a **Caution—Radioactive Material** label and a **Radioactive Waste Pick-up Receipt**



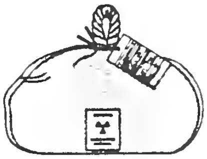
Large Animals

- Use a MPW box, follow MPW packaging and radioactive waste disposal procedures
- Attach a **Caution—Radioactive Material** label and a **Radioactive Waste Pick-up Receipt**



Animal Bedding and/or Solid Excreta

- Double bag in plastic bags
- Use absorbent material, if necessary
- Close each bag separately. Twist top of bag; bend twisted portion to form a loop and close with a bag closure tie
- Attach a **Caution—Radioactive Material** label
- Attach a **Radioactive Waste Pick-up Receipt**
- Refrigerate or freeze waste if held for more than 24 hours



Disposal Information

For Assistance About:	Call:	At:
Waste Pick-up from Workplace	Radioactive Waste Service Information needed to request waste pick-up: • Authorized user name and RSB ID number • Location—building and room • Type of waste—liquid, solid, LS vials, etc. • Radionuclide(s) and estimated amount of activity for each • Special instructions	6-4451
Assistance	Radiation Safety Branch	6-5774

May, 1994

Laboratory Safety Supplies Available Through the NIH Self-Service Stores in Building 10 or 35

Antimicrobial Liquid Soap: A liquid soap in a convenient pump-type dispenser. Soap contains softeners and emollients to help prevent dry skin.

18 oz. pump-type dispenser #6508-00-L01-5805

Apron: PVC/Nylon apron. Heavy duty. Yellow. Suitable for use with most corrosives and general lab activities. Provides additional personal protection when worn over lab coat.

One size # 8415-00-L01-0235

[Betadine] Povidone-Iodine Topical Solution: For topical use in the event of exposure to blood and body fluids & bloodborne pathogens.

One pint bottle # 6505-00-958-1730

Biological Sample Carrier: Polycarbonate carrier. Hinged lid with silicone gaskets. Autoclavable. For use in transporting biological materials, safely. Has stainless steel handle.

$14^{1/2}$ x 7 x 6 $^{3/4}$ inches # 6640-00L01-1350

Biohazard Labels: International biohazard symbol printed on blaze orange labels. When using these labels, please include the hazard identity, your name and date. Please remove label(s) when no longer needed.

1" x 3" labels/500 per roll	#7530-00L0-72375
$2^{1/4}$" x 4" labels/ 50 per package	#7530-00L0-72376

Bottle Carriers: Impact resistant bottle carriers for hazardous chemicals. For use in temporary storage and transport of laboratory chemicals.

Four liter carrier	#664000L01-1343
Pint carrier/ holds six one-pint bottles	#664000L01-1340

Bottle Top Dispenser: Dispenses 2 to 10 ml directly from the bottle. Reduces risk of spills. Purchased only through Delpro.

Made by Brinkman	# 6640-00-L01-2080

Chemical Splash Goggles: An improved chemical splash goggle that combines splash protection and impact resistance. Lenses are specially treated for UV resistance and to prevent fogging. The goggles can be easily worn over eyeglasses. Meets ANSI Z87.1-1989

One pair per box	# 4240-01-136-3637

Coveralls--Breathable Woven: Full-body protection for blood and body fluid protection. New woven fabric is breathable, yet resistant to fluid exposures. Elasticized sleeves, zipper front. Manufactured by Kimberly-Clark.

Medium	# 8415-00L-01-1825
Large	# 8415-00L-01-1826

Face Shield: Impact resistant polycarbonate face shield

Visor	# 4240-01-214-5772
Headgear	# 4240-01-214-5771

Gown--Disposable Polyethylene : Resistant to blood and body fluids. Closed front. Knit sleeves. Ties in back.

30 Gowns per case # 6532-01-306-2709

Kevlar Cut Resistant Gloves: Woven kevlar material provides protection against slashing cuts and injuries to hands. Gloves can be worn over disposable gloves to combine cut resistance with barrier protection. Washable. Grip has raised plastic dots to improve grip. Gloves do not ensure protection against point-puncture injuries.

Medium	# 6515-00L03-244
Large	# 6515-00I03-245

Leather Palm Work Gloves: General duty gloves that provide additional protection when working with glass, etc.

Per Pair # 8415-00-634-4656

Mucous Membrane Protection: Acetate face shield attached to surgeon-type mask. Disposable. Barrier protection for blood and body fluid procedures. Masks are not suitable for situations in which respiratory protection is required--call your ICD Safety and Health Specialist-6-2346.

Ten masks per bag #6515-00-L01-3935

N-DEX Nitrile Gloves: Provide a greater degree of protection when working with potential pathogens. Form fitting gloves resist tears better than conventional PVC or Latex gloves. The gloves also provide a degree of protection from various laboratory chemicals. A chemical resistance guide (available from your ICD Safety and Health Specialist, phone 6-2346) lists specific applications.

X-Small	# 6515-00-L03-2565
Small	# 6515-00-L03-2566
Medium	# 6515-00-L03-2567
Large	# 6515-00-L03-2568

Nitrile Gloves: Re-usable, chemically resistant nitrile gloves. Green, heavy duty. Consult the manufacturer's chemical resistance guide (available from your ICD Safety and Health Specialist, phone 6-2346) for specific applications.

Small	#6515-00L0-32441
Medium	#6515-00L0-32442
Large	#6515-00L0-32443

Non-prescription Safety Glasses: Adjustable impact resistant safety glasses for routine lab activities. Suitable for general wear when the potential for a splash hazard is low. Polycarbonate lenses are treated for UV and scratch resistance. Meets ANSI Z87.1-1989.

One pair per box	# 4240-00-L01-5809

Pipet-Aids: Conventional and portable vacuum assisted pipet aids.

Conventional vacuum pump type	# 6640-01-137-9255
Rechargeable portable type	# 6640-010195-5299

Pipet Bulb: Conventional pipet bulb. For any pipet.

Each # 6640-00-051-9490

Pipet Helper: Adjustable pipet aid. Adjusts from 0.1 to 100 ml volumes. Chemically resistant.

Made by Brinkman # 6640-00-L01-5165

Pipettor--Hand Held: Piston-type pipet aid for pipets up to 2 ml.

Made by Bel-Art # 6640-01-127-0822

Pipet Tray: Polypropylene tray. Ideal for use inside biological safety cabinets to safely collect pipets and other contaminated items. Chemically resistant. Autoclavable.

Made by Nalge # 6640-01-122-1771

Sharps Safe: Puncture resistant plastic boxes with lids. Suitable for use with needles, razor blades, broken pipets and other sharp objects. Remember, close and seal each container when it is 3/4 full and place in a MPW Box for disposal. Sharps Safes must not go into general trash.

Six liter	# 6530-01-294-2865
Eleven liter	# 6530-01-274-5099
Twenty-one liter	# 6530-01-196-0283

Sleeves--Disposable Polyethylene: Resistant to blood and body fluids. 18 inches long. Covers arm from wrist to elbow. Elastic cuffs.

100 Sleeves per case. # 6532-01-305-9059

Sleeves--for Micro Centrifuges: Aluminum sleeves and plastic caps for use in Eppendorf Micro Centrifuge units. Fits both 0.5 and 1.5 ml tubes

Per kit # 6640-00-L01-7332

Terry Cloth Autoclave Gloves: Heat resistant gloves. Ideal for handling items into and out of the autoclave.

Per pair # 8415-01-145-3287

Vacuum Filters: Filters for the protection of vacuum systems. Package includes instructions on proper use/applications.

Three filters per package (Gelman)	#6640-00-L01-2200
Ten filters per package (Whatman)	#6640-00-L01-2219

For additional assistance in selecting or obtaining safety supplies for your research--please contact your ICD Safety and Health Specialist at 6-2346.

Notes